PB89-166581

Treatability Potential for EPA (Environmental Protection Agency) Listed Hazardous Wastes in Soil

Texas Univ. at Austin

Prepared for

Robert S. Kerr Environmental Research Lab.
Ada, OK

Mar 89

U.S. Department of Commerce
National Technical Information Service
NTIS

PB89-166581

EPA/600/2-89/011
March 1989

TREATABILITY POTENTIAL FOR EPA LISTED HAZARDOUS WASTES IN SOIL

by

Raymond C. Loehr
Environmental Engineering Program
The University of Texas at Austin
Austin, Texas 78712

Project CR-812819

Project Officer

Scott G. Huling
Extramural Activities and Assistance Division
Robert S. Kerr Environmental Research Laboratory
Ada, Oklahoma 74820

ROBERT S. KERR ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ADA, OKLAHOMA 74820

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA/600/2-89/011

2.

3. RECIPIENT'S ACCESSION NO.
PB89 166581/AS

4. TITLE AND SUBTITLE
TREATABILITY POTENTIAL FOR EPA LISTED HAZARDOUS WASTES IN SOIL

5. REPORT DATE
March 1989

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Raymond C. Loehr

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental and Water Resources Engineering
University of Texas at Austin
Austin, TX 78712

10. PROGRAM ELEMENT NO.
ABWDIA/CBWDIA

11. CONTRACT/GRANT NO.
CR-812819

12. SPONSORING AGENCY NAME AND ADDRESS
Robert S. Kerr Environmental Research Laboratory
U.S. Environmental Protection Agency
P. O. Box 1198
Ada, OK 74820

13. TYPE OF REPORT AND PERIOD COVERED
Final Report (9/85 - 2/89)

14. SPONSORING AGENCY CODE
EPA/600/15

15. SUPPLEMENTARY NOTES
Project Officer: Scott G. Huling, FTS: 743-2313 (405) 332-8800, X313

16. ABSTRACT

This study developed comprehensive screening data on the treatability in soil of: (a) specific listed hazardous organic chemicals, and (b) waste sludge from explosives production (K044) and related chemicals. Laboratory experiments were conducted using two soil types, an acidic soil, and a slightly basic sandy loam soil. The experiments were developed to evaluate: (a) the relative toxicity of the chemicals and waste using the MICROTOX bioassay method, (b) the degradation potential of the chemicals and waste in the soils, (c) the adsorption characteristics of the chemicals in the two soils, and (d) the toxicity reduction that occurred during degradation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT
RELEASE TO THE PUBLIC

19. SECURITY CLASS (This Report)
UNCLASSIFIED

20. SECURITY CLASS (This page)
UNCLASSIFIED

21. NO. OF PAGES
134

22. PRICE

NOTICE

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under Cooperative Agreement CR-812819 to The University of Texas at Austin. It has been subjected to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

EPA is charged by Congress to protect the Nation's land, air, and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: (a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment; (b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; (c) develop techniques for predicting the effect of pollutants on groundwater, soil, and indigenous organisms; and (d) define and demonstrate the applicability and limitations of using natural processes, indigenous to the soil and subsurface environment, for the protection of this resource.

Soil treatment systems that are designed and managed based on a knowledge of soil-waste interactions may represent a significant technology for simultaneous treatment and ultimate disposal of selected hazardous wastes in an environmentally acceptable manner. Decisions pertaining to which wastes and chemicals are amenable to this technology must take into account: 1) the long-term uncertainties associated with the land disposal option; 2) the goal of managing hazardous wastes in an appropriate manner; and 3) the persistence, toxicity, mobility, and propensity to bioaccumulate hazardous wastes and their hazardous constituents. There is currently a lack of scientifically derived fate and transport information for the wide range of hazardous chemicals for which such decisions can be made. This report presents information pertaining to the quantitative evaluation of the treatment potential in soil of specific listed hazardous organic chemicals as identified by the United States Environmental Protection Agency (EPA), and waste sludge from explosives production and its related chemicals.

Clinton W. Hall

Clinton W. Hall
Director
Robert S. Kerr Environmental
Research Laboratory

ABSTRACT

This study developed comprehensive screening data on the treatability in soil of: (a) specific listed hazardous organic chemicals, and (b) waste sludge from explosives production (KO44) and related chemicals. Laboratory experiments were conducted using two soil types, an acidic soil (Mississippi soil) with less than one percent organic matter, and a slightly basic sandy loam soil (Texas soil) containing 3.25% organic matter. These experiments evaluated the: (a) relative toxicity of the chemicals and waste using the Microtox© bioassay method, (b) degradation of the chemicals and waste in the soils, (c) adsorption characteristics of the chemicals in the two soils, and (d) toxicity reduction that occurred during degradation.

The major conclusions were:

1. The chemical structure of the compounds evaluated affected their relative toxicity. With chlorophenols, the relative toxicity was related to the position of the chlorine group on the phenol ring. The order of relative toxicity was para>meta>ortho. The same order appeared to occur for methylphenols and nitrophenols. The chemical substituted on the phenol ring appeared to have an effect on toxicity. Nitro-substituted phenols appeared to be less toxic than the methyl- or chloro-substituted phenols. Mixing of the chemicals with the soils did not affect the relative toxicity of the chemicals in the two soils.
2. Data characterizing the chemical loss in the soil and in the water soluble fraction (WSF) extracted from the soil as well as the toxicity reduction in the WSF could be represented satisfactorily by either first or zero order

kinetics. In most cases, the data were represented by either kinetic parameter with high correlation coefficients.

3. The rates of chemical loss were higher in the Texas soil. Chlorophenols with chlorine substituted in the meta position had greater half-lives and lower loss rates. Chemicals with a nitro group substituted in the phenol ring appeared to have a lower loss rate.
4. The Freundlich equation described the adsorption of most of the chemicals with the two soils satisfactorily. The values of the Freundlich constant (K_f) for the chemicals in the two soils were different. For the acid extractables, the K_f values generally were greater in the Mississippi soil. For the amines and alcohols, the K_f values were greater in the Texas soil.
5. The loss of the applied chemical in the soil and in the WSF as well as the reduction of the WSF toxicity were compared for nine of the chemicals. The chemical loss in the WSF was about 1.5 times faster than the chemical loss in the soil. The WSF toxicity decreased at about the same rate as the WSF chemical concentration. No enhanced mobilization of the applied chemical occurred during degradation.

CONTENTS

SECTION **PAGE**

NOTICE ii

FOREWORD iii

ABSTRACT iv

FIGURES x

TABLES xi

ACKNOWLEDGEMENTS xiv

SECTION 1. INTRODUCTION 1
  Scope of Study 1
  Designated Chemicals and Waste 2

SECTION 2. CONCLUSIONS 5
  A. Chemical Toxicity 5
  B. Degradation Studies 6
  C. Adsorption Studies 6
  D. Toxicity Reduction 7
  E. Munitions Chemicals and Wastes 8

SECTION 3. GENERAL MATERIALS AND METHODS 8
  Chemicals 9
  Soil 9
  Instrumentation 11
    Gas Chromatography 11
    High Pressure Liquid Chromatography 13
  QA/QC Procedures 13
  Toxicity 13

SECTION 4. RELATIVE TOXICITY AND CHEMICAL LOADING 14
  Objectives 14
  Background 14
    Microtox 15
    EC_{50} Evaluation 16
  Acceptable Loadings 19
  Conclusions 27

SECTION 5. DEGRADATION STUDIES 29
  Introduction 29
  Materials and Methods 31

CONTENTS, Continued

SECTION PAGE

Results 37
  Recovery Efficiency 37
  Kinetic Parameters 37
Conclusions 46

SECTION 6. ADSORPTION EXPERIMENTS 49
Introduction 49
  Adsorption Equilibria 50
  Soil Organic Carbon 51
  Soil pH 52
Materials and Methods 54
  Adsorption Method 54
  Stock Solutions 54
  Standard Solutions 54
  Soil Moisture 56
  Soil:Solution Ratio 56
  Solute Stability 58
  Other Factors 58
  Data Analysis 59
Results 60
Conclusions 67

SECTION 7. TOXICITY REDUCTION 69
Approach 69
Results 70
  Chemical Loss in Soil 70
  WSF Chemical Loss 72
  WSF Toxicity Reduction 76
  Comparison of Chemical Losses and Toxicity Reduction 76
Conclusions 81

SECTION 8. MUNITIONS WASTES AND CHEMICALS 82
Relative Toxicity and Loading Evaluation 82
Adsorption 83
  Methods 83
  Results 84
Degradation Studies 87
Munitions Wastewater Treatment Sludge 87
  Nitrogen and COD 87
  Metals 91
  GC/MS Analysis 91
  Relative Toxicity 91
Conclusions 95

SECTION 9. REFERENCES 97

CONTENTS, Continued

SECTION PAGE

APPENDIX A. THE MICROTOX© TOXICITY ASSAY USED IN THIS STUDY ... 101
  Introduction ... 101
  Evaluation of Chemical Loss Using Microcosms ... 101
  Water Extraction of Microcosms ... 101
  Toxicity Assay ... 102
  EC_{50} Determination ... 102
  Assay Procedure ... 102
  Chemical Loading on Soil ... 103
  Microtox© Analysis ... 104

APPENDIX B. QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES ... 107
  Toxicity ... 107
  Degradation Studies ... 107
  Adsorption Studies ... 109
  QA/QC For Analytical Instruments ... 111

APPENDIX C. VOLATILIZATION ESTIMATES ... 114

APPENDIX D. PUBLICATIONS ... 117

FIGURES

NUMBER PAGE

1. Schematic of the Phenol Ring and Possible Substitution Positions 18
2. Loss of Phenol in Texas Soil at 20° C 42
3. Loss of Phenol in Mississippi Soil at 20° C 42
4. Loss of 2,6-Dichlorophenol in Texas Soil at 20° C 43
5. Loss of 2,6-Dichlorophenol in Mississippi Soil at 20° C 43
6. Adsorption of 2,3-Dichlorophenol and Toluenediamine in Texas Soil at 20° C 61
7. Adsorption of 2,3-Dichlorophenol and Toluenediamine in Mississippi Soil at 20° C 62
8. Loss of Chemical in Two Experiments When the Higher Loading Rates Were Used 74
9. Loss of Chemical in the WSF at Two Loading Rates 75
10. Toxicity Reduction in the WSF from the Soil Microcosms 78
11. Comparison of Chemical Loss in the Soil and the WSF for Phenol and Eight Chlorophenols 79
12. Decrease of Soil and WSF Chemical Concentration and of WSF Toxicity as a Function of Time-Degradation Study of 2,4-Dichlorophenol 80
13. Comparison of the WSF Chemical Loss and the WSF Toxicity Reduction For Phenol and Eight Chlorophenols 80
14. Loss of 2,6-Dinitrotoluene in Texas and Mississippi soils at 20° C 89
15. Wastewater Treatment Process Flow Diagram for the Holston Army Ammunition Plant 90

B-1. Representative Recovery Data for Phenol in Texas Soil 110

B-2. Representative Recovery Data for o-Cresol in Texas Soil 110

B-3. Representative Recovery Data for 2,4-Dichlorophenol in Texas Soil 111

TABLES

NUMBER PAGE

1. Chemicals That Were Evaluated in This Study 3
2. The Hazardous Waste and Related Chemicals That Were Evaluated 4
3. Characteristics of Soils Used in This Study 10
4. Operating Conditions for the Gas Chromatographic Analysis of Compounds in Methylene Chloride 12
5. EC_{50} for Chemicals Evaluated in This Study 17
6. Relative Toxicity of Chlorinated Phenols 20
7. Relative Toxicity of Non-Chlorinated Phenols 20
8. Comparative Relative toxicity of Chloro-, Methyl, and Nitrophenols 22
9. Method to Determine Acceptable Initial Chemical Loadings 22
10. Acceptable Non-Inhibitory Loading Rates -- Texas Soil 23
11. Acceptable Non-Inhibitory Loading Rates -- Mississippi Soil 24
12. Comparative Acceptable Loading Rates -- Chlorinated Phenols 25
13. Experimental Procedures Used in the Degradation Studies 33
14. Chemicals Whose Soil Concentration Was Evaluated Using Shake Extraction 34
15. Shake Extraction Procedure 34
16. Recovery Efficiencies for Specific Chemicals (%) 38
17. Acceptable and Actual Loading Rates Used in the Degradation Studies (mg/kg of Soil) 39
18. Loss Rates, Correlation Coefficients and 95% Confidence Intervals for Specific Chemicals -- Texas Soil 40
19. Loss Rates, Correlation Coefficients and 95% Confidence Intervals for Specific Chemicals -- Mississippi Soil 41

TABLES, Continued

NUMBER **PAGE**

20. Chemical Half-Lives in Texas and Mississippi Soils (days) 45

21. Effect of Substitution Position on Degradation Rates -- Chlorinated Phenols 47

22. Effect of Substitution Position on Degradation Rates -- Non-Chlorinated Phenols 47

23. Comparative Degradation Rates of Chloro-, Methyl- and Nitrophenols.... 48

24. Procedure for Determining Solubility Limit of an Organic Compound In Water 55

25. List of Maximum Solubilities (20° C) in Water Determined as Part of the Adsorption Studies 55

26. Procedure for Measuring Soil Moisture Content 56

27. Procedure for Determination of Optimum Soil:Solution Ratio 57

28. Batch Sorption Isotherm Data -- Texas Soil -- Freundlich Equation Parameters 63

29. Batch Sorption Isotherm Data -- Mississippi Soil -- Freundlich Equation Parameters 64

30. Chemical Concentration Range Evaluated During the Batch Adsorption Experiments -- Texas Soil 65

31. Chemical Concentration Range Evaluated During the Batch Adsorption Experiments -- Mississippi Soil 66

32. Comparison of Freundlich Adsorption Coefficients (K_f) for the Texas and Mississippi Soils 68

33. Chemical Loss in Soil -- Kinetic Parameters 71

34. Loss of Water Extractable Chemical -- Kinetic Parameters 73

35. WSF Toxicity Reduction -- Kinetic Parameters 77

36. EC_{50} Data and Acceptable Loading Rates for Munitions Manufacturing Chemicals 83

TABLES, Continued

NUMBER **PAGE**

37. Chemical Concentration Range Evaluated During the Batch Adsorption Experiments -- Munitions Chemicals.......85

38. Freundlich Isotherm Data -- Texas and Mississippi Soils -- 2,4- and 2,6-Dinitrotoluene.......85

39. Adsorption Data for TNT, HMX and RDX in Texas Soil at 20° C.......86

40. Adsorption Data for TNT, HMX and RDX in Mississippi Soil at 20° C.......86

41. Loading Rate and Recovery Efficiency Data from the Munitions Chemical Degradation Studies.......88

42. Chemical Loss Rate Data for 2,6-Dinitrotoluene and TNT in the Degradation Studies.......88

43. Nitrogen and COD Concentrations of Munitions Waste Sludge.......91

44. Metals in Munitions Sludge and Sludge Filtrate.......92

45. GC/MS Analysis of Munitions Sludge.......93

46. Munitions Waste Toxicity Data -- Undiluted Samples.......95

B-1. Accuracy Data: Recovery Efficiencies (%) for Specific Chemicals As Determined From Day Zero Degradation Study Experiments.......108

B-2. Precision Analysis Data: Texas Soil Sorption Data.......112

C-1. Loss of Methanol and 1-Butanol in Volatilization Experiments.......115

ACKNOWLEDGEMENTS

This report represents the hard work and dedication of the undergraduate students, graduate students, staff and faculty who assisted in this project. The contribution of the following individuals deserves specific recognition and is greatly appreciated:

Barnes Bierck
Srinivasa Dasappa
Carol English
David Erickson
Lisa Gilmour-Stallsworth
Nadine Gordon
Frank Hulsey
R. Krishnamoorthy
Michael McFarland
Joseph F. Malina, Jr.
Wan Nam-Koong
Lynn Sanders
Karen Spaniel
John Stephenson
Eric White
Chun Yoon

We also appreciate the assistance of the EPA-RSKERL project officers, Mr. John E. Matthews and Mr. Scott G. Huling, who had responsibility for the project throughout its duration.

SECTION 1

INTRODUCTION

SCOPE OF STUDY

This study was designed to provide comprehensive screening data on the treatability in soil of: (a) EPA listed hazardous organic chemicals, and (b) a specific hazardous waste and related chemicals. The results of the study provide data that can be used when permitting decisions are made related to: (a) management of spills, (b) remediation of contaminated soils, and (c) the use of land as a waste management alternative. The degradation and partitioning data can be used as input to predictive models that estimate the movement of chemicals in the unsaturated zone of the soil. Examples of such models include RITZ (Regulatory and Investigative Treatment Zone Model)(1, 2), VIP (Vadose Zone Interactive Processes Model)(3, 4), and KOPT (Kinematic Oily Pollutant Transport Model(5).

These models were developed to understand the treatment potential of organic chemicals in soil. The models integrate the processes that affect chemicals in soil (degradation and partitioning) so that an assessment can be made of the extent to which protection of human health and the environment occurs. The understanding that results from the use of such models allows the identification of chemicals and wastes that require control to reduce or eliminate their hazard potential prior to application to soil.

Laboratory studies were conducted to determine: (a) degradation kinetics, (b) sorption, (c) toxicity of the chemicals and waste, and (d) the reduction in toxicity that occurs during degradation. The results of these studies are discussed in subsequent sections.

DESIGNATED CHEMICALS AND WASTES

The chemicals and specific waste that were part of this study are identified as hazardous wastes under CFR Sections 261.32 and 261.33. These chemicals can be expected to be components of many industrial compounds and wastes that enter the soil from spills and inadequately sealed impoundments (pits, ponds and lagoons) and as part of wastes applied to operating land treatment units.

The chemicals that were evaluated are identified in Table 1. The specific hazardous waste, and chemicals related to that waste, that were evaluated are noted in Table 2.

Samples of the explosives waste sludge (KO44) and the chemicals TNT, RDX, and HMX were obtained with the help of the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA). A sample of wastewater treatment sludge resulting from the manufacture and processing of explosives was obtained from the Holston Army Ammunition Plant with the assistance of USATHAMA. This material was stored at 4° C until required for analysis and use.

TABLE 1. CHEMICALS THAT WERE EVALUATED IN THIS STUDY

Compound	Formula	EPA Hazardous Waste Number
Acid Extractables		
Phenol	C_6H_6O	U188
o-Cresol	C_7H_8O	U052
p-Cresol	C_7H_8O	U052
m-Cresol	C_7H_8O	U052
2-Chlorophenol	C_6H_5ClO	U048
3-Chlorophenol	C_6H_5ClO	NOS
4-Chlorophenol	C_6H_5ClO	NOS
2,3-Dichlorophenol	$C_6H_4Cl_2O$	NOS
2,4-Dichlorophenol	$C_6H_4Cl_2O$	U081
2,5-Dichlorophenol	$C_6H_4Cl_2O$	NOS
2,6-Dichlorophenol	$C_6H_4Cl_2O$	U082
3,4-Dichlorophenol	$C_6H_4Cl_2O$	NOS
2,4,5-Trichlorophenol	$C_6H_3Cl_3O$	U230
2,4,6-Trichlorophenol	$C_6H_3Cl_3O$	U231
Pentachlorophenol	C_6HCl_5O	U242
2,4-Dimethylphenol	$C_8H_{10}O$	U101
2-Methyl-4-Chlorophenol	C_7H_7ClO	NOS
3-Methyl-4-Chlorophenol	C_7H_7ClO	U039
3-Methyl-6-Chlorophenol	C_7H_7ClO	NOS
p-Nitrophenol	$C_6H_5NO_3$	U170
2,4-Dinitrophenol	$C_6H_4N_2O_5$	P048
4,6-Dinitro-o-Cresol	$C_7H_6N_2O_5$	P048
Thiophenol	C_6H_6S	U014
Amines		
Diphenylamine	$C_{12}H_{11}N$	X016
m-Phenylenediamine	$C_6H_8N_2$	X017
Toluenediamine	$C_7H_6(NH_2)_2$	U221
Brucine	$C_{23}H_{26}N_2O_4$	P018
Alcohols		
Isobutyl alcohol	$C_4H_{10}O$	U140
Allyl alcohol	C_3H_6O	P005
Propargyl alcohol	C_3H_4O	P102
1-Butanol	$C_4H_{10}O$	U031
2,3-Dichloropropanol	$C_3H_6Cl_2$	X006
Methanol	CH_4O	U154
Other		
Carbon disulfide	CS_2	P022
2-Nitropropane	$C_3H_7NO_2$	U171
Thiourea	CH_4N_2S	U219

TABLE 2. THE HAZARDOUS WASTE AND RELATED CHEMICALS THAT WERE EVALUATED

Specific Hazardous Waste

KO44 -- Wastewater treatment sludge from the manufacturing and processing of explosives

Explosive and Munitions Manufacturing Chemicals

Compound	Formula	EPA Hazardous Waste Number
2,4-Dinitrotoluene	$C_7H_6N_2O_4$	U105
2,6-Dinitrotoluene	$C_7H_6N_2O_4$	U106
TNT (2,4,6-Trinitrotoluene)	$C_7H_5N_3O_6$	–
RDX$^+$	$C_3H_6N_6O_6$	–
HMX^{++}	$C_4H_8N_8O_8$	–

$^+$ *RDX = Hexahydrotrinitrotriazine*

$^{++}$ *HMX = Cyclotetramethylenetetranitramine*

SECTION 2

CONCLUSIONS

The major results were the following:

A. CHEMICAL TOXICITY

1. The Microtox© biological assay represents an appropriate method with which to evaluate the EC_{50} toxicity of a chemical or waste.
2. Comparison of the EC_{50} data indicated that: (a) the alcohols were less toxic than the acid extractable compounds, and (b) within chemical categories, there were considerable differences in relative toxicity.
3. The chemical structure of the compounds evaluated affected the relative toxicity of a compound. With chlorophenols, the relative toxicity was related to the substitution position of the chlorine group on the phenol ring. The order of relative toxicity was para>meta>ortho. The EC_{50} data suggested that the same order occurred for methylphenols and nitrophenols.
4. The chemical that was substituted on the phenol ring appeared to have an effect on toxicity. Nitro-substituted phenols, even when substituted in the para position, appeared to be less toxic than the methyl- or chloro- substituted phenols.
5. When the chemicals were mixed with two different soils, and the EC_{50} value of the water soluble fraction (WSF) of the soil mixtures was measured, the values also indicated that chemicals with the chlorine in the para position had the greater toxicity. Mixing of the chemicals with the soils did not affect the relative toxicity of the chemicals in the two soils.
6. In general, the acceptable non-inhibitory chemical loading rates for the Mississippi soil were lower than those for the Texas soil. There was no consistent pattern for the differences.

B. DEGRADATION STUDIES

7. The chemical or waste loading procedure (Table 9, Section 4) resulted in chemical loadings that did not inhibit the non-acclimated organisms in the laboratory microcosms, except in one case (4,6-Dinitro-o-Cresol). This procedure provided a good estimate of initial, acceptable chemical loadings that can be used in laboratory degradation studies.

8. Both zero and first order kinetics provided adequate representation of the data. For most of the chemicals, the data could be fit to either kinetics with high correlation coefficients.

9. The rates of chemical loss were higher in the Texas soil than in the Mississippi soil. There did not appear to be any pattern to the differences in rates in the two soils.

10. Chlorophenols with the chlorine substituted in the meta position had greater half-lives and therefore lower chemical loss rates. This was particularly evident with the mono-, di-, and trichlorophenols in the Texas soil.

11. Chemicals that had a nitro group substituted on the phenol ring appeared to have a lower loss rate.

C. ADSORPTION STUDIES

12. The Freundlich equation described the adsorption of the chemicals on the two soils satisfactorily, with high correlative coefficients, except for a few chemicals.

13. The range of chemical concentrations evaluated ranged from the low mg/l concentrations to near or at saturation concentrations, and for most chemicals covered two to three orders of magnitude. For these

concentration ranges, a linear adsorption relationship, i.e., n = 1, did not occur.

14. The values of the Freundlich constant (K_f) for the chemicals in the two soils were different. For the acid extractables, the K_f values generally were greater in the Texas soil which had the higher pH and the greater organic carbon content. For the amines and alcohols, the K_f values were greater in the Mississippi soil, which had the lower pH and the lower organic carbon content.

D. TOXICITY REDUCTION

15. Two loading rates, the Texas soil, and nine chemicals (phenol and eight chlorinated phenols) were used in this study. Both first and zero order kinetics satisfactorily fit the water soluble fraction (WSF) chemical loss data and the toxicity reduction data.

16. The higher chemical loading rates resulted in higher chemical concentrations in the WSF and higher WSF toxicities at the beginning of the experiments.

17. The higher chemical loading rates generally resulted in slower chemical losses (higher half lives) and slower toxicity reduction. However, at both loading rates for each chemical, the chemicals were degraded and the toxicity was reduced. No differences due to the loading rates were apparent in zero order kinetics.

18. The loss of the chemicals in the WSF was about 1.5 times faster than the loss of the chemical in the soil.

19. The WSF toxicity for each chemical decreased as the soil chemical and the WSF chemical concentrations decreased.

20. The WSF toxicity decreased at about the same rate as the WSF chemical concentration when the data for all nine chemicals were compared.

21. No enhanced mobilization of the applied chemicals occurred as the degradation and detoxification occurred.

22. No water soluble toxic products appeared to be formed as the chemicals were degraded in the soil.

E. MUNITIONS CHEMICALS AND WASTES

23. The Freundlich equation described the sorption of 2,4- and 2,6-Dinitrotoluene in the two soils satisfactorily. It did not do so for TNT, RDX, or HMX.

24. No loss of 2,4-Dinitrotoluene occurred over a 47-day study even though the loading rate used was determined to be acceptable using procedures discussed in Section 4. Degradation loss rates were obtained for 2,6-Dinitrotoluene and TNT. First order kinetics were a better representation for TNT than were zero order kinetics.

25. The half life of TNT in the Mississippi soil was shorter, and the loss faster, than in the Texas soil. No difference in the loss rates in the two soils for 2,6-Dinitrotoluene was apparent.

26. The sludge resulting from the manufacture and processing of explosives contained: (a) high concentrations of nitrogen and COD, (b) concentrations generally less than 10 mg/l for heavy metals, and (c) no TNT, RDX or HMX.

27. The munitions sludge had a high toxicity as measured by the Microtox© procedure. The constituents causing the relative toxicity were in the soluble phase of the sludge.

SECTION 3

GENERAL MATERIALS AND METHODS

CHEMICALS

The chemicals evaluated in this study were identified in Section 1. To the extent possible, analytical grade chemicals were used in the experiments and as spikes and controls in the analytical procedures. The chemicals were purchased either from Aldrich Chemicals or Sigma Chemicals. Specific explosive and munitions manufacturing chemicals were obtained from sources identified in Section 8.

SOILS

The intent of this study was to provide comprehensive screening data on the treatability of specific chemicals and a hazardous waste in soil. The characteristics of the soil will affect the degradation, sorption, and treatment potential and two soils with different characteristics were used. One was an acid soil with a low organic content and the other was a basic soil with a higher organic content and cation exchange capacity (CEC).

The acid soil was obtained from an area near Wiggins, Mississippi, and was supplied by researchers at Mississippi State University. This soil is referred to as Mississippi soil in this report. The characteristics of this soil are presented in Table 3. The analyses were conducted by staff at Mississippi State University using appropriate methods(6).

The basic soil was obtained from an area near Austin, Texas, that, to the knowledge of the personnel of this project, had not been exposed to industrial chemicals or wastes. This soil is referred to as Texas soil. The characteristics of the soil are presented in Table 3. The analyses were conducted by staff at the soil testing and characterization laboratory, Texas A&M University, College Station, Texas.

Because independent laboratories provided separate analyses of the soils, the data in Table 3 are not always directly comparable. However, the data provide pertinent information on the important characteristics of the two soils.

Both soils had initial microorganism counts that were typical for an agricultural soil with an active microbial population.

TABLE 3. CHARACTERISTICS OF SOILS USED IN THIS STUDY

Characteristic	Mississippi Soil	Texas Soil
Texture	sandy loam	sandy silt loam
Classification	Typic Paleudults	Mollisal, Cumulic
pH	4.8	7.8
CEC (meq/100 g)	6.35	10.8
Organic Carbon	0.94%	3.25%
Sodium	0.02 meq/100 g	0.3 meq/L$^+$
Potassium	0.07 meq/100 g	3.6 meq/L
Calcium	0.29 meq/100 g	9.5 meq/L
Magnesium	0.06 meq/100 g	1.3 meq/L
Hydrogen	5.9 meq/100 g	–
Carbonate (CO_3)	–	0.0 meq/L
Bicarbonate (HCO_3)	–	7.2 meq/L
Sulfate	–	1.9 meq/L
Chloride	–	2.8 meq/L
Particle Size Fraction (%)		
Sand	68.0	61.5
Silt	23.4	31.1
Clay	8.6	7.4
Moisture Content by Weight (%)		
1/3 atmosphere	12.4	17.0
15 atmosphere	8.2	6.2

$^+$ -- *Saturated paste extract*

After the soils were received at the Environmental and Water Resources Engineering Laboratory at The University of Texas, they were air dried, sieved and stored at 4° C in the dark. Prior to each experiment, soil samples were taken for use, the moisture content increased to near saturation, and the indigenous organisms allowed to establish equilibrium concentrations.

INSTRUMENTATION

The principal analytical instruments used were the Microtox© analyzer, the gas chromatograph (GC), and the high pressure liquid chromatograph (HPLC). The Microtox© unit, which was used for chemical toxicity evaluation, is discussed in detail in Section 4. The following describes the operational procedures employed for the GC and HPLC analyses.

Gas Chromatography

Two types of gas chromatographs were used to quantify chemical concentrations. The choice of GC depended on the volatility of the compound and the solvent used with the compound.

Compounds extracted in Methylene Chloride were analyzed by capillary column gas chromatography. The analytical procedure followed is outlined in EPA-SW 846 (Method 8040)(7). The method consisted of injection of a 1 µL sample into the gas chromatograph (Hewlett Packard Model 5890A) which was equipped with an electronic integrator (Hewlett Packard Model 3392A), a methyl silicone capillary column, and a flame ionization detector. The chromatographic system was calibrated using an internal standard technique each day.

The operating conditions of the capillary column gas chromatograph are presented in Table 4. The initial temperature and temperature progress rate were selected based on the retention time of the test compound and the internal standard. The integrator options were set to minimize the tailing of the chromatographic peaks, which affected the peak area calculations. The initial temperature was between 30° C and 100° C depending on the retention time of the compound, the temperature progress rate was between 15° C and 30° C/minute, and the final temperature was 180° C. A two-minute final time was utilized at the termination of each run in order to ensure that the column was clean.

TABLE 4. OPERATING CONDITIONS FOR THE GAS CHROMATOGRAPHIC ANALYSIS OF COMPOUNDS IN METHYLENE CHLORIDE

Condition		Description
Capillary Column		methyl silicone (dimensions: 5m length x 0.53 mm I.D. x 2.65 μm film thickness)
Temperature	*Initial*	30° C-100° C at 0 minutes with progress rate of 15-30° C per minute.
	Final	180° C at 2 minutes
	Injection Port	200° C
	Detector	250° C
Detector		Flame Ionization
	Hydrogen Gas Rate	30 mL/minute
	Air Rate	400 mL/minute
Carrier Gas	*Helium Rate*	20 mL/minute

Compounds extracted in either water or Methanol (e.g., alcohols) were quantified on a packed column gas chromatograph (Tracor 550) equipped with a six-foot column packed with 5% Carbowax 1500, 80/100 Carbopack K-C (Supelco, Inc.). The GC was operated isothermally at 130° C with a flame ionization detector. The electronic output signal was converted to concentration units by interfacing the GC with an integrator (Spectraphysics Model 4290). The GC was calibrated by the internal standard technique outlined in Method 8040 (EPA-SW846)(7).

High Pressure Liquid Chromatography

Phenolic compounds contained in filtered water (i.e., as part of the sorption study, Section 6) and the compounds of low volatility (Brucine, Thiourea, RDX, HMX and TNT) were analyzed by high pressure liquid chromatography (HPLC).

The HPLC (Waters Associates Model 440) was operated at room temperature and utilized a C-18 reversed phase column. Aqueous solutions (50 µL) of various samples were eluted with 50:50:0.1 of acetonitrile:deionized distilled water:acetic acid solution. The UV absorption detector wavelength was 254 nm. The flow rate and chart speed were maintained at 3.0 ml/min and 0.1 inch/min respectively. The attenuation varied from 0.01 to 2.0 depending on the relative compound absorption at 254 nm. Duplicate samples were analyzed to ensure instrument accuracy.

Standard solutions for each compound were run prior to sample analysis for external standard calibration. The peak height on the chart paper for each standard solution was measured and linear regression analysis (Lotus 1-2-3, IBM/PC) used to determine the relationship between peak height (cm) and concentration (mg/L). Comparison of peak heights of samples to peak heights determined for standard solutions allowed estimation of sample chemical concentrations.

QA/QC PROCEDURES

Care was taken to assure that sound, representative data were obtained. The specific quality assurance and quality control procedures that were followed and information on precision and accuracy are presented in Appendix B.

TOXICITY

The relative toxicity of the chemicals and the specific waste as well as of the water soluble fraction of soil-chemical mixtures was measured by the Microtox© system. Details of the system and how it was used are presented in Section 4.

SECTION 4

RELATIVE TOXICITY AND CHEMICAL LOADING

OBJECTIVES

A major objective of this study was to obtain information on the degradation kinetics of these chemicals and specific waste in soil. To do so, it was important that the chemicals be in the soil in concentrations that would not be toxic or inhibitory to the soil microorganisms. Therefore, it was necessary to determine: (a) the relative toxicity of the chemicals and specific waste prior to adding them to the soils, and (b) the mass loading rates of the chemicals that would not be inhibitory.

The relative toxicity tests that were conducted were not intended to provide information on toxicity from a human health or safety or from an environmental standpoint. Rather, these tests were used as a relative toxicity screening method. Such tests also can be used to identify the relative toxicity reduction that occurs when chemicals and waste are managed by the land treatment process.

BACKGROUND

The usual procedures to quantify toxicity of a chemical are toxicity assays which measure the effect of the chemical on a test species under specified test conditions. The toxicity of a chemical is proportional to the severity of the chemical on the monitored response of the test organism(s). Toxicity assays utilize test species that include rats, fish, invertebrates, microbes and seeds. The assays may use single or multiple species of test organisms. The need to unequivocally measure the effect of the toxicant on the monitored activity has favored the use of a single specie as the test organism in a toxicity assay.

Toxicity assays using bacteria as the test organism are gaining popularity due to their rapidity, ease in handling, cost effectiveness and the use of a statistically significant number of test organisms(8, 9). The Microtox© assay is a microbial assay used for toxicity measurement, hazard assessment, and quantitative-structure activity relationship (QSAR) studies of environmental pollutants(8-11).

Although no single bioassay procedure can provide a comprehensive toxicity evaluation of a chemical, a valid toxicity screening test can provide information about the relative toxicity of a compound and can help predict non-inhibitory chemical application rates. The Microtox© system is a relatively simple, rapid and inexpensive test and was used as the toxicity screening method in this project. The use of the Microtox© procedure to screen and predict the treatability potential of waste in soil has been evaluated and found to be satisfactory(12, 13).

Microtox©

The Microtox© system is a standardized toxicity test system which utilizes marine luminescent bacteria (*Photobacterium phosphoreum*) as indicator organisms. Bioluminescence of this test organism depends on a complex chain of biochemical reactions involving the luciferin-luciferase system. Chemical inhibition any of the involved biochemical reactions causes a reduction in bacterial luminescence. The Microtox© toxicity assessment considers the physiological effect of a toxicant, and not just mortality.

The system utilizes an instrumental approach in which the indicator organisms are handled as chemical reagents. Suspensions of about one million bioluminescent organisms are "challenged" by addition of serial dilutions of an aqueous sample. A temperature controlled photometric device quantifies the light output in each suspension before and after sample addition. Reduction of light output reflects physiological inhibition which indicates presence of toxic constituents in the sample. The small sample volume required (~10 mL) is a positive aspect of the system.

The instrument used in this project was the Beckman Microtox© Model 2055 Toxicity Analyzer System. Except for slight modifications, procedures indicated in the

Microtox© System Operating Manual[14] were followed. A summary of the procedure that was used is included in Appendix A.

EC_{50} Evaluation

The EC_{50} is the concentration of a chemical that causes a 50% decrease of light produced by luminescent bacteria in the Microtox© test. The method provides for simultaneous testing of a control and dilutions of a chemical. The percent light decrease after 5 minutes is plotted against sample concentration. The concentration that diminishes the light output by 50% is designated the EC_{50} under the defined conditions of exposure time and test temperature. When calculating EC_{50} data, responses for all concentrations are normalized against blank responses by multiplying the initial light output of each concentration by the mean blank ratio for time *t*. This normalization corrects for effects of light drift and offsets in light output due to dilution. An explanation of the analytical and calculation methods used to obtain EC_{50} data is presented in Appendix A.

EC_{50} evaluations for the specific chemicals are presented in Table 5. Results for the munitions chemicals and sludge are presented in Section 8. These EC_{50} values represent values for the chemicals and sludge as a solution of the chemical and of the as-received sludge. The values are not comparable to the EC_{50} values that might occur in a soil-chemical or soil-sludge mixture. These EC_{50} values are useful as: (a) a relative screening evaluation for the chemicals to estimate which chemicals have a greater toxicity potential, and (b) input to decisions about concentrations that can be applied to soil that will be non-inhibitory to the soil microorganisms.

In Table 5, the 95% confidence values (95% CI) of the EC_{50} values also are presented. The 95% CI was calculated in a standard statistical manner. Specific details about the procedure are presented in Section 5. The 95% CI identifies the

TABLE 5. EC_{50} DATA FOR CHEMICALS EVALUATED IN THIS STUDY

Compound	EC_{50} Values (mg/l) Value	EC_{50} Values (mg/l) 95% CI
Acid Extractables		
Phenol	26.7	25.4-28.1
o-Cresol	22.4	21.8-23.0
p-Cresol	1.1	1.07-1.16
m-Cresol	5.8	5.6-6.1
2-Chlorophenol	16.1	15.1-17.1
3-Chlorophenol	3.8	3.4-4.3
4-Chlorophenol	1.0	0.9-1.0
2,3-Dichlorophenol	4.8	4.7-5.0
2,4-Dichlorophenol	2.8	2.7-2.9
2,5-Dichlorophenol	8.2	7.8-8.6
2,6-Dichlorophenol	15.9	14.8-17.1
3,4-Dichlorophenol	0.5	0.4-0.5
2,4,5-Trichlorophenol	0.9	0.8-1.0
2,4,6-Trichlorophenol	9.3	8.6-10.1
Pentachlorophenol	1.1	1.1-1.2
2,4-Dimethylphenol	3.8	3.4-4.3
2-Methyl-4-Chlorophenol	1.1	1.06-1.14
3-Methyl-4-Chlorophenol	0.3	0.2-0.3
3-Methyl-6-Chlorophenol	5.8	5.7-6.0
p-Nitrophenol	7.0	6.7-7.3
2,4-Dinitrophenol	13.5	11.0-16.6
4,6-Dinitro-o-Cresol	10.5	9.1-12.1
Thiophenol	2.8	2.6-3.1

Compound	EC_{50} Values (mg/l) Value	EC_{50} Values (mg/l) 95% CI
Amines		
Diphenylamine	2	1.9-2.1
m-Phenylenediamine	112	99-122
Toluenediamine	44	41-47
Brucine	213	197-230
Alcohols		
Isobutyl alcohol	1740	1590-1920
Allyl alcohol	1120	960-1320
Propargyl alcohol	106	99-115
1-Butanol	2400	2180-2640
2,3-Dichloropropanol	120	104-140
Methanol	>84,500	–
Other		
Carbon disulfide	30	27-32
2-Nitropropane	49	41-59
Thiourea	4530	4050-5080

range of values within which the estimated true mean EC_{50} value should occur with the probability of error being 0.05. The relatively narrow 95% CI values indicate the low scatter of the data obtained.

Low EC_{50} values indicate a higher toxicity potential and the higher EC_{50} values indicate a low toxicity. In Table 5, the EC_{50} data indicate that phenol (EC_{50} = 26.7) has a lower toxicity potential than p-Cresol (EC_{50} = 1.1).

The comparison of the EC_{50} data (Table 5) indicates that: (a) the alcohols are less toxic than the acid extractables, and (b) within particular categories, there are considerable differences in relative toxicity. It appeared that there was a relationship between the relative toxicity and the chemical structure of the chemical. This was explored using EC_{50} values for several of the acid extractables.

In discussing these relationships, an understanding of the structure of the phenol compound and where substitutions can occur is helpful. Figure 1 indicates the basic structure of phenol and indicates that substitutions can occur at five locations that can be identified by number or name. Thus, a 2-Chlorophenol indicates a chlorine compound is substituted at the 2- or ortho position. A p-nitrophenol indicates that a nitro- (NO_2) group is located at the 4- or para position.

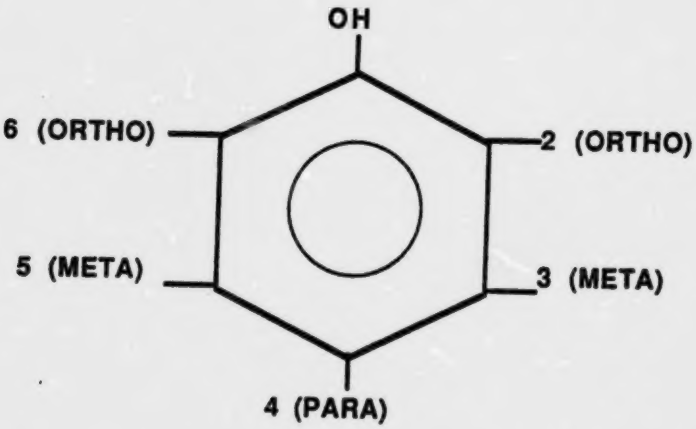


Figure 1. Schematic of the Phenol Ring and Possible Substitution Positions

Data for fourteen chlorinated phenols were evaluated to identify whether a relationship between chemical structure and relative toxicity existed in this study. The EC_{50} values for these compounds provide a reasonable data base to consider the effect of chemical structure. When the EC_{50} values were compared (Table 6), the relative toxicity of these compounds appeared related to the substitution position of the chlorine group on the phenol ring. The order of toxic potential was para>meta>ortho. The order was particularly evident with the mono- and di-chlorophenols.

This order also appeared when the methylphenols were compared (Table 7). There were not enough data to infer that the same order occurred with nitrophenols, although it was suggested (Table 7).

The chemical compound that is substituted on the phenol ring also appeared to have an effect on toxicity (Table 8). Nitro-substituted phenols, even when substituted in the para position, appear to be less toxic than the methyl- or chloro-substituted phenols.

While it appears that the chemical structure of a compound, such as a substituted phenol, affects the relative toxicity of the compound, this study was not designed to determine such effects or the reason differences occur. The effect of substitution position and chemical may be due to the influence of physicochemical, electronic and/or steric properties of the chemical .

ACCEPTABLE LOADINGS

Microbial degradation is the major organic removal mechanism in the soil. Therefore, microbial inhibition by a chemical or waste can be a limiting factor when chemicals are added to the soil. A chemical and waste loading determination protocol for land treatment demonstrations to estimate the non-inhibitory loading has been proposed(12, 13). This protocol combines the leaching potential of the waste and the toxicity of the leachate to arrive at a non-inhibitory loading. In this study, the acceptable loading rates were determined by following this protocol which is outlined

TABLE 6. RELATIVE TOXICITY OF CHLORINATED PHENOLS

Compound	EC_{50} Value (mg/l)	Substitution Position
2-Chlorophenol	16.1	ortho
3-Chlorophenol	3.8	meta
4-Chlorophenol	1.0	para
2,3-Dichlorophenol	4.8	ortho, meta
2,4-Dichlorophenol	2.8	ortho, para
2,5-Dichlorophenol	8.2	ortho, meta
2,6-Dichlorophenol	15.9	ortho, ortho
3,4-Dichlorophenol	0.5	meta, para
2,4,5-Trichlorophenol	0.9	ortho, para, meta
2,4,6-Trichlorophenol	9.3	ortho, para, ortho
Pentachlorophenol	1.1	all
2-Methyl-4-Chlorophenol	1.1	ortho, para
3-Methyl-4-Chlorophenol	0.3	meta, para
3-Methyl-6-Chlorophenol	5.8	meta, ortho

TABLE 7. RELATIVE TOXICITY OF NON-CHLORINATED PHENOLS

Compound	EC_{50} Value (mg/l)	Substitution Position
Methylphenols		
o-Cresol (2-Methylphenol)	22.4	ortho
p-Cresol (4-Methylphenol)	1.1	para
m-Cresol (3-Methylphenol)	5.8	meta
2,4-Dimethylphenol	3.8	ortho, para
Nitrophenols		
p-Nitrophenol	7.0	para
2,4-Dinitrophenol	13.5	ortho, para

in Table 9. The logic behind this procedure is that other studies(12, 13) have shown that the detoxification of water soluble organics in soil did not occur or proceeded very slowly when the EC_{50} value of the initial mixture was less than 20%.

When determining an acceptable chemical loading, it is assumed that any toxicity that results is due to the added chemical and not the soil. This assumption was evaluated and the toxicity of the WSF of the Texas and Mississippi soils was determined using the Microtox© procedure. No toxicity of either soil was found.

The acceptable loading rates that resulted when the specific chemicals were mixed with two soils are noted in Tables 10 and 11. These acceptable loading data should be viewed as initial screening data for degradation and land treatment demonstration studies. The protocol employs only an acute toxicity testing. The soil:solution ratio used (1:4) is low and simulates only short-term leaching effects. In the degradation studies (Section 5), the actual loading rates used were equal to or less than those noted in Tables 10 and 11.

The loading limit of several of the alcohols was not determined. This was because the large quantities of such soluble chemicals that were needed saturated the soil and caused nonrepresentative land treatment conditions.

The effect of chemical structure on the acceptable loading rate also was evaluated. The chlorinated phenol data (Table 12) indicated that the chemicals with chlorine in the para position generally required the lower non-inhibitory loading rates. The mixing of the chemicals with the two different soils did not appear to affect the relative toxicity order of the chemicals discussed earlier.

In general, the acceptable loading rates for the Mississippi soil were lower than those for the Texas soil. There was, however, no consistent pattern for the differences. Some of the rates were close and some differed by an order of magnitude. The experiments were not planned to investigate the reasons for differences with different

TABLE 8. COMPARATIVE RELATIVE TOXICITY OF CHLORO-, METHYL- AND NITROPHENOLS

Compound	EC_{50} Value (mg/l)
3-Chlorophenol	3.8
m-Cresol (3-Methylphenol)	5.8
p-Nitrophenol	7.0
p-Cresol (4-Methylphenol)	1.1
4-Chlorophenol	1.0
2,4-Dinitrophenol	13.5
2,4-Dimethylphenol	3.8
2,4-Dichlorophenol	2.8

TABLE 9. METHOD TO DETERMINE ACCEPTABLE INITIAL CHEMICAL LOADINGS

1. Prepare several different ratios of a waste-soil mixture or a chemical-soil mixture. This results in different loading rates.
2. Obtain a water soluble fraction (WSF) for each mixture, i.e., loading rate.
3. Determine the EC_{50} for each WSF.
4. Determine relative toxicity units using the following equation: $TU = 400/EC_{50}$.*
5. Prepare a log-log plot of TU values versus loading rate.
6. The interception point for 20 toxicity units (20TU) is the lower loading limit for the soil. Since there can be a window of acceptable lower loading rates, a value of twice the lower limit is identified as the upper limit for this acceptable window.*

* *See Appendix A for discussion of these items.*

TABLE 10. ACCEPTABLE NON-INHIBITORY LOADING RATES -- TEXAS SOIL

Compound	Loading Rate[+] (mg/kg of soil)
Acid Extractables	
Phenol	720
o-Cresol	490
p-Cresol	100
m-Cresol	124
2-Chlorophenol	380
3-Chlorophenol	120
4-Chlorophenol	88
2,3-Dichlorophenol	130
2,4-Dichlorophenol	90
2,5-Dichlorophenol	300
2,6-Dichlorophenol	630
3,4-Dichlorophenol	30
2,4,5-Trichlorophenol	40
2,4,6-Trichlorophenol	300
Pentachlorophenol	30
2,4-Dimethylphenol	88
2-Methyl-4-Chlorophenol	48
3-Methyl-4-Chlorophenol	18
3-Methyl-6-Chlorophenol	150
p-Nitrophenol	250
2,4-Dinitrophenol	1380
4,6-Dinitro-o-Cresol	350
Thiophenol	160
Amines	
Diphenylamine	230
m-Phenylenediamine	7900
Toluenediamine	1080
Brucine	3600
Alcohols	
Isobutyl alcohol	++
Allyl alcohol	++
Propargyl alcohol	8200
1-Butanol	++
2,3-Dichloropropanol	1050
Methanol	++
Other	
Carbon disulfide	400
2-Nitropropane	1620
Thiourea	6580

[+] *lower loading rate as determined using the procedure in Table 9*

[++] *not determined, see text*

TABLE 11. ACCEPTABLE NON-INHIBITORY LOADING RATES -- MISSISSIPPI SOIL

Compound	Loading Rate$^+$ (mg/kg of soil)
Acid Extractables	
Phenol	320
o-Cresol	250
p-Cresol	50
m-Cresol	130
2-Chlorophenol	290
3-Chlorophenol	60
4-Chlorophenol	45
2,3-Dichlorophenol	60
2,4-Dichlorophenol	45
2,5-Dichlorophenol	30
2,6-Dichlorophenol	50
3,4-Dichlorophenol	23
2,4,5-Trichlorophenol	30
2,4,6-Trichlorophenol	215
Pentachlorophenol	55
2,4-Dimethylphenol	45
2-Methyl-4-Chlorophenol	38
3-Methyl-4-Chlorophenol	10
3-Methyl-6-Chlorophenol	140
p-Nitrophenol	45
2,4-Dinitrophenol	270
4,6-Dinitro-o-Cresol	26
Thiophenol	330
Amines	
Diphenylamine	110
m-Phenylenediamine	*
Toluenediamine	1400
Brucine	3200
Alcohols	
Isobutyl alcohol	++
Allyl alcohol	++
Propargyl alcohol	5100
1-Butanol	++
2,3-Dichloropropanol	700
Methanol	++
Other	
Carbon disulfide	1540
2-Nitropropane	1170
Thiourea	910

$^+$ *lower loading rate as determined using the procedure in Table 9*

$^{++}$ *not determined, see text*

* *chemical deterioration of this chemical in this soil prevented evaluation of mass loading data*

TABLE 12. COMPARATIVE ACCEPTABLE LOADING RATES -- CHLORINATED PHENOLS

Compound	Loading Rate (mg/kg) Texas Soil	Loading Rate (mg/kg) Mississippi Soil	Substitution Position
2-Chlorophenol	380	290	ortho
3-Chlorophenol	120	60	meta
4-Chlorophenol	88	45	para
2,3-Dichlorophenol	130	60	ortho, meta
2,4-Dichlorophenol	90	45	ortho, para
2,5-Dichlorophenol	300	30	ortho, meta
2,6-Dichlorophenol	630	50	ortho, ortho
3,4-Dichlorophenol	30	23	meta, para
2,4,5-Trichlorophenol	40	30	ortho, para, meta
2,4,6-Trichlorophenol	300	215	ortho, para, meta
Pentachlorophenol	30	55	all
2-Methyl-4-Chlorophenol	48	38	ortho, para
3-Methyl-4-Chlorophenol	18	10	meta, para
3-Methyl-6-Chlorophenol	150	140	meta, ortho

soils. Therefore, it was not possible to identify the fundamental factors causing the observed differences. However, there are several possibilities.

The concept of determining an acceptable chemical loading for a soil is to identify an application rate or range of rates that will ensure degradation of waste without inhibiting the microorganisms in the soil. The chemical loading determination procedure consists of two aspects: (i) quantity of the chemical partitioning into the water soluble fraction (WSF) under standard test conditions, and (ii) toxicity of WSF.

The first aspect is a function of sorption characteristics of the chemical under the test conditions. The second aspect depends on the relative toxicity of the chemical, the impact of soil characteristics on the toxicity, and the method used to measure toxicity.

The sorption of a chemical in soil depends on: (i) soil characteristics such as pH, organic content, particle size distribution, and presence of other chemicals, (ii) chemical characteristics such as solubility, partition coefficient, and pK value, and (iii) test conditions such as soil:solution ratio, test temperature and agitation.

The sorption data obtained in this study are presented in Section 6 and there are differences in the sorption characteristics of the two soils. Sorption increases the persistence of a chemical in the soil and reduces the migration potential of the chemical. Therefore, a chemical that has greater sorption characteristics will be more tightly bound to the soil and less likely to be in solution and therefore in the WSF. The basis of the procedure to estimate the acceptable chemical or waste loading rate (Table 9) is the assumption that the WSF of the chemical or waste poses the immediate and significant threat to soil microorganisms and to groundwater.

The results that were obtained were similar to those obtained by other researchers. Sims(15) reported waste mass loading data for petroleum and wood-preserving wastes with clay loam and sandy loam soils. The same procedure (Table 9) was used to obtain the data. In the clay loam soil, higher chemical loadings were possible than with the sandy loam soil. This was attributed to the higher adsorption and hence lower leaching of the wastes in clay loam soil. Pentachlorophenol (PCP) waste showed a lower loading than the other wastes containing creosote and polynuclear aromatic hydrocarbons (PAH's). This may have been due to the higher toxicity of PCP using the Microtox© assay.

The pH of the Microtox© method also may have had an effect on speciation of the chemicals that were available for sorption and the resultant relative toxicity. The acceptable pH of the sample in the Microtox© assay is the range of 6.0-8.0. The pH of the samples analyzed in this study was within this range. The chemicals evaluated have pKa values that range widely. As an example, the pKa of pentachlorophenol (PCP) is about 5.0 while that of phenol is about 9.3. When a sample is adjusted to the

Microtox© pH range (6.0-8.0), the speciation will vary. For instance, at this pH range, PCP would be completely dissociated, whereas the dissociation of phenol would be minimal.

The method (Table 9) used to identify acceptable, non-inhibitory chemical and waste loading rates is conservative in that acclimation of the organisms to the waste or chemicals is not considered. In the field the actual non-inhibitory waste and chemical loading rates to the soil may be higher than those noted in this study since microbial acclimation will occur with time.

The higher the chemical or waste loading that can be successfully treated on the land, the more economical will be the land treatment option. Huddleston et al.(16) reported increased soil respiration rates and waste removal rates with an increase in oil waste application in land treatment investigations. The microbial acclimation may significantly increase the degradation of a waste at high waste application concentrations(15, 17). However, the method in Table 9 does result in an initial chemical and waste loading that will not inhibit the soil microorganisms. Such loadings will allow the soil treatability of chemicals and wastes to be determined.

CONCLUSIONS

1. The Microtox© biological assay represents an inexpensive and expedient method with which to evaluate the EC_{50} toxicity of a chemical or waste. This method was used to estimate the EC_{50} values of specific chemicals and to estimate the non-inhibitory soil loadings of the chemicals and waste that were evaluated.

2. The comparison of the EC_{50} data indicated that: (a) the alcohols were less toxic than the acid extractable compounds, and (b) within chemical categories, there were considerable differences in relative toxicity.

3. The chemical structure of the compounds evaluated affected the relative toxicity of a compound. With chlorophenols, the relative toxicity was related to the substitution position of the chlorine group on the phenol ring. The order of relative toxicity was para>meta>ortho. The EC_{50} data suggested that the same order occurred for methylphenols and nitrophenols.

4. The chemical that was substituted on the phenol ring appeared to have an effect on toxicity. Nitro-substituted phenols, even when substituted in the para position, appeared to be less toxic than the methyl- or chloro-substituted phenols.

5. When the chemicals were mixed with two different soils, and the EC_{50} value of the water soluble fraction (WSF) of the soil mixtures was measured, the values indicated that chemicals with the chlorine in the para position had the greater toxicity. Mixing of the chemicals with the soils did not affect the relative toxicity of the chemicals in the two soils.

6. In general, the acceptable non-inhibitory chemical loading rates for the Mississippi soil were lower than those for the Texas soil. There was no consistent pattern for the differences. The differences were likely due to different sorption characteristics of the chemicals in the two soils.

SECTION 5

DEGRADATION STUDIES

INTRODUCTION

These experiments were conducted to determine the removal kinetics of the designated chemicals and wastes (Table 1) in soil. Several of the chemicals could not be evaluated because of chemical reactions in the soils which made analytical detection impossible. These were Diphenylamine, m-Phenylenediamine and Thiophenol.

Biodegradation is believed to be the most important removal mechanism for organic compounds in soil systems. Biodegradation of organics is accomplished in a series of biochemical reactions through which a parent compound is changed or transformed to organic and inorganic end products. Complete degradation is the term used to describe the process whereby constituents are mineralized to inorganic end products, including carbon dioxide, water, and inorganic nitrogen, phosphorus, and sulfur compounds.

Aerobic soil bacteria possess the ability to biochemically catalyze the oxidation of organic compounds. For this reason, and because the zone of incorporation at land treatment sites generally is aerobic, the protocol used in this study allowed aerobic conditions and aerobic biodegradation reactions to occur.

In the mixed microbial population of soil systems, one metabolic group of microorganisms may partially metabolize a compound and may furnish a suitable growth substrate for another group. Organic compounds also may be partially degraded or transformed to organic intermediates that may be recalcitrant and/or toxic.

The primary goal of biodegradation testing is to obtain an overall estimate of the rate at which a compound will biodegrade in a soil environment. While few compounds appear in the environment in pure form, a common approach for studying removal rates of organic compounds has been to evaluate individual compounds. Although this approach provides an understanding of the removal rates for specific compounds, it is recognized that during actual land treatment chemicals normally are applied as mixtures. Interactions between compounds in a mixture within the soil matrix may promote or inhibit their removal from soil.

In this study, the chemicals were evaluated as individual compounds and the data should be understood in that context.

Methods used to evaluate the biodegradation of organics in the environment commonly use indirect measures such as oxygen consumption, CO_2 evolution, and dissolved organic carbon (DOC) loss, to assess the persistence of compounds in test environments, to determine chemical loss rates and to predict the relative importance of biodegradation.

While these procedures provide a qualitative assessment of biodegradation, they do not determine quantitative rates of degradation for specific constituents. Such chemical specific rates are essential for the assessment of compound fate and transport as well as for risk analyses. For quantitative assessment of the rate of biodegradation of an individual constituent in a soil system, it is necessary to measure changes in parent compound concentration with time, and the loss of a chemical due to methods other than biodegradation. In addition, the immobilization potential (partitioning into soils, liquid, and gaseous phases) provides additional information for assessing the soil treatment potential of hazardous constituents.

In this study, no distinction was made between specific loss mechanisms. Thus removal rates can be due to biodegradation, chemical degradation, hydrolysis, photolysis and volatilization. The chemicals that were evaluated did not have a high volatilization potential and volatilization was not considered an important removal mechanism in these degradation experiments. The logic for this statement is presented in Appendix C. Thus, the degradation rates presented in this section were not corrected for volatilization.

MATERIALS AND METHODS

In this study, the rate of degradation was experimentally determined by measuring the difference between the amount of compound initially added to a soil and that which was recovered after specified time intervals. The general protocol used to determine these differences is presented in Table 13. The protocol notes that the soils should be maintained at a moisture content which is about 80% of field capacity. This will provide adequate moisture for the biodegradative reactions but avoid saturated conditions. For the two soils that were used, the equivalent of 80% field capacity was a moisture content of about 12% for the Mississippi soil and about 16% for the Texas soil.

Soil used in this experimental program: (a) had not had previous exposure to industrial chemicals or wastes, and (b) did not receive any pretreatment such as soil amendments or specially acclimated biological cultures prior to these experiments. Thus, the naturally occurring soil microbial consortium was responsible for the bioremediated removal of the chemicals.

Chemical mass loadings were determined as part of the toxicity screening evaluations (Section 4). These determinations ensured that the loadings at which the chemicals were applied did not inhibit soil microbial activity. Soil pH was not adjusted nor were supplementary organic substrates used. The control beaker (blank) was carried through the experimental procedure to ensure quality control of the instrumental analysis.

Various techniques can be used to apply test compounds to soil in laboratory-scale studies. When the test compounds were not highly water soluble, the test compound was dissolved in a small aliquot of solvent before application into a soil. In these experiments, the chemicals were added to the soil as a solution of water or of Methylene Chloride depending on their solubility characteristics.

To minimize the possible toxic effect of the Methylene Chloride, a small volume (100 µL) of the solvent, which contained an appropriate amount of chemical for a specific initial concentration, was applied to each soil sample and mixed thoroughly immediately after application with glass stirrers. Each beaker contained a glass stirrer to prevent possible cross-contamination. A 20 µL microdispenser was utilized to distribute the 100 µL solvent/chemical at five different points on the surface of the soil sample. The Methylene Chloride also was added in the above volume to the control beakers. Prior to applying the aluminum foil cover, a brief time was allowed for volatilization of the Methylene Chloride.

As noted in Table 13, at selected time intervals the concentration of the remaining chemical in a set of beakers was determined. The time intervals were based on estimates of the half-life of the chemical and were chosen to provide at least five data points to establish the removal rates. At these sampling periods, a sample set (four beakers -- one blank and three with chemical) was taken from the constant temperature room and extracted for sixteen hours with either Methylene Chloride for phenolic compounds or Methanol for amines (including Brucine and Thiourea) in a Soxhlet extraction apparatus (Method 3540)(7).

The extract was concentrated in a Kuderna-Danish extraction unit attached to a three-ball Snyder column (Method 3540)(7). The concentration step was conducted in a water bath maintained at 60° C for phenolic compounds and at 78° C for amines. The concentrated extracts were dried by passing them through disposable sodium sulfate columns and then refrigerated at 4° C until analysis by gas chromatography (GC) or high pressure liquid chromatography (HPLC).

Several of the chemicals were extracted using water as a solvent and the shake

TABLE 13. EXPERIMENTAL PROCEDURES USED IN THE DEGRADATION STUDIES

Each experiment consisted of eight sample sets. Each sample set contained four beakers (triplicates for a sample and one blank). The experimental procedure for each chemical was as follows;

(a) Place 10 g of air-dried soil, which has been kept at 4° C, into each 150 ml beaker and adjust the soil moisture content to 80% of field capacity with distilled deionized water. Place a glass stirrer in each beaker. Record all weights, including beaker, soil, water and glass stirrer.

(b) Mix soil and water thoroughly, cover the beakers with aluminum foil and place the beakers in the dark at 20° C. The cover minimizes water loss and the possible addition of contaminated dust.

(c) Wait for 10 days to allow soil microorganisms to equilibrate to experimental conditions.

(d) Prepare solutions of the test chemicals so that 100 μL of the solution gives the desired mass loading rate. The mass loadings are based on toxicity screening results.

Add 100 μL of solution into each sample beaker using a 20 μL micropipet. Do this five times to distribute the solution to five points on the soil surface and mix thoroughly. Return the beakers to the constant temperature room.

(e) Sample beakers should be arranged so that the chemical solution is added to the day 0 beaker last. For example, if samples are scheduled at day 0, 2, 4, 8, 16, 24, 32, and 64, the order for adding a chemical to soil is sample sets for day 64, 32, 24, 16, 8, 4, 2 and day 0.

(f) The beakers are incubated in the dark to prevent photodegradation of the added chemicals.

(g) During the study, adjust the moisture content in each beaker weekly and maintain between 60% and 80% of field capacity.

(h) Sacrifice sample sets at the selected time intervals for test chemical analysis.

(i) When experimental data show that the chemical remaining in the soil is below GC or HPLC detection limits, the remaining sample beakers can be discarded.

extraction procedure. The compounds extracted in this manner are noted in Table 14 and the extraction procedure is summarized in Table 15.

DATA ANALYSIS

Biodegradation information can be used to identify the rate of loss of an organic chemical in a soil. Such information results from treatability studies such as those conducted in this study. Biodegradation rates were determined experimentally by applying the chemical of interest to a soil microcosm and monitoring concentration over time. A plot of the disappearance of a constituent originally present in the chemical/soil mixture versus treatment time provided the following: (a) the type of reaction (generally zero or first order), (b) the reaction rate constants for the zero or first order reactions, and (c) the half-life ($t_{1/2}$) time of each constituent of concern.

TABLE 14. CHEMICALS WHOSE SOIL CONCENTRATION WAS EVALUATED USING SHAKE EXTRACTION

Compound	Solvent Used
Methanol	water
1-Butanol	water
Isobutyl Alcohol	water
2,3-Dichloropropanol	Methylene Chloride
2-Nitropropane	water
Allyl Alcohol	water
Propargyl Alcohol	water

TABLE 15. SHAKE EXTRACTION PROCEDURE

Each experiment consisted of eight sample sets. Each sample set contained four beakers (triplicates for a sample and one blank). The experimental procedure for each chemical was as follows.

(a) Place 40 g of air-dried soil, which has been kept at 4° C, into each of the 200 ml mason jars and adjust the soil moisture content to 80% of field capacity with distilled deionized water. Record all weights, including beaker, soil, water and glass stirrer.

(b) Mix soil and water thoroughly then cap reactors with screw-on top and place in 20° C incubator.

(c) Wait for 10 days to allow soil microorganisms to equilibrate to experimental conditions.

(d) Prepare solutions of the test chemicals so that 100 μL of the solution gives the desired mass loading rate. The mass loadings are based on toxicity screening results.

Add 100 μL of solution into each sample beaker using a 20 μL micropipet and mix thoroughly. Return the reactors to incubator.

(e) On day of analysis, four reactors are removed. Each is filled with 200 milliliters of the appropriate extracting solvent. The reactors are sealed with the gas tight cap and placed on the shaker apparatus.

(f) The shaker apparatus is operated at 250 rpm for one hour. After this period, the reactors are allowed to settle for 15 to 30 minutes.

(g) The supernatant from the reactors is decanted and centrifuged at 5000 rpm for 30 minutes.

(h) After centrifugation, the supernatant is filtered using a 0.45 μm pore size filter and the filtrate stored at 4° C until analysis on the gas chromatograph.

First and zero order kinetic relationships were used to model data from the degradation studies. The following describes both kinetic relationships.

The expression for first order kinetics is:

$$-dC/dt = kC \tag{1}$$

where C is the chemical concentration (mg of compound/kg of soil), t is time (days), and k is the first order kinetic constant (day $^{-1}$). The integrated form of Equation 1 is:

$$C = C_0 \exp(-kt) \tag{2}$$

where C_0 is the initial concentration of chemical.

Taking the natural logarithm of Equation 2 results in:

$$\ln C = (\ln C_0) - kt \tag{3}$$

Using linear regression, the relationship between ln C and t (time) data allows determination of the loss coefficient k (which is the slope of ln C versus t curve).

Half life ($t_{1/2}$) is defined as the time required for the amount of chemical to decrease to half of its initial value. Half life values based on first order kinetics are obtained by rearranging Eqn. 3:

$$t_{1/2} = \ln 2/k \tag{4}$$

The common expression for zero order kinetics is:

$$-dC/dt = K \tag{5}$$

where K is the zero order kinetic constant (mg/Kg/day). The integrated form of Equation 5 is:

$$C = (C_0) - Kt$$

The zero order kinetic constant K may be estimated by analyzing C versus t data by linear regression.

Data obtained from each sampling interval were used to calculate both first order and zero order kinetic parameters. Half-life data were calculated based on first order kinetics. Total loss rate data (mg chemical/kg dry soil/day) were reported for zero order kinetics.

The zero and first order kinetic parameters and the correlation coefficient were obtained from a least squares fit of the data. To estimate the 95% confidence interval for the data, the standard deviation and the student t statistic were computed based on the data and used as follows(18):

$$95\%\ \mathrm{CI} = (k \text{ or } K) \pm \frac{t \cdot s}{(ss_x)^{1/2}}$$

where: k or K = least squares calculated first or zero order kinetic parameter,

t = student t statistic for the data and 95% confidence,

s = sample standard deviation, and

ss_x = sum of squares for the data.

Recovery efficiency data for each chemical were acquired and used to determine the chemical concentration that actually remained in the soil. The day zero extraction data identified the extraction efficiency of the chemical under the conditions of that specific experiment. It was assumed that the day zero extraction efficiency was constant throughout the specific experiment. The recovery efficiencies were used to calculate the actual soil concentration prior to extraction as follows. Assume that: (a) the recovery efficiency was 50% as shown by the day zero data and (b) the extracted soil concentration for the chemical at sampling time three was 100 mg/kg soil. The actual recovery efficiency corrected chemical concentration at this sampling time was calculated to be 200 mg/kg (extraction concentration divided by extraction efficiency). The recovery efficiency corrected chemical concentrations were used in all calculations to determine the kinetic relationships and constants.

Because of time constraints, the appropriate analytical protocol for measuring Carbon Disulfide (CS_2) was not developed. Thus, this chemical is not included as part of these experiments.

RESULTS

Recovery Efficiency

Recovery efficiency data for the selected chemicals are given in Table 16. There were differences in recovery efficiencies for the two soils but there were no consistent patterns. The differences may be due to the different sorption characteristics of the soils with specific chemicals.

Kinetic Parameters

The loading rates for the chemicals to the soil were at or below the acceptable loading rates determined as described in Section 4 (Table 17). In some cases, the actual loading rates were slightly above the lower acceptable loading rate but were within the acceptable loading rate window. In one case (4,6-Dinitro-o-Cresol in Mississippi soil) (Table 17), the actual loading rate inadvertently was considerably higher than the acceptable loading rate. Except in the latter case, the loading rates should not have been such to inhibit the microorganisms in the two soils during the degradation experiments.

The first and zero order rate constants, correlation-coefficients, and 95% confidence intervals for the selected chemicals in Texas and Mississippi soils are given in Tables 18 and 19 respectively. Chemical loss curves illustrative of the type obtained in this study are shown in Figures 2-5.

In reviewing the kinetic parameters, it should be recalled that a small half-life and a large zero order constant indicate a faster degradation rate. For example, in the Texas soil, p-Cresol had a higher degradation rate and was removed faster in the microcosms than was phenol.

In general, high correlation coefficients were obtained with the data. Higher correlation coefficients were obtained with the acid extractables in both soils. Based on the correlation coefficient data and a visual inspection of the chemical loss curves,

TABLE 16. RECOVERY EFFICIENCIES+ FOR SPECIFIC CHEMICALS (%)

Compound	Texas Soil	Mississippi Soil
Acid Extractables		
Phenol	88	78
o-Cresol	57	63
p-Cresol	78	97
m-Cresol	81	88
2-Chlorophenol	23	25
3-Chlorophenol	94	85
4-Chlorophenol	97	85
2,3-Dichlorophenol	115	104
2,4-Dichlorophenol	82	92
2,5-Dichlorophenol	88	84
2,6-Dichlorophenol	80	71
3,4-Dichlorophenol	115	104
2,4,5-Trichlorophenol	82	92
2,4,6-Trichlorophenol	88	84
Pentachlorophenol	115	106
2,4-Dimethylphenol	80	81
2-Methyl-4-Chlorophenol	91	101
3-Methyl-4-Chlorophenol	99	99
3-Methyl-6-Chlorophenol	100	100
p-Nitrophenol	110	110
2,4-Dinitrophenol	95	132
4,6-Dinitro-o-Cresol	84	100
Amines		
Toluenediamine	100	72
Brucine	65	100
Alcohols		
Isobutyl alcohol	92	94
Allyl alcohol	119	110
Propargyl alcohol	95	97
1-Butanol	90	89
2,3-Dichloropropanol	100	86
Methanol	100	106
Other		
2-Nitropropane	60	55
Thiourea	88	93

+ *Average of results from triplicate samples*

TABLE 17. ACCEPTABLE AND ACTUAL LOADING RATES USED IN THE DEGRADATION STUDIES (mg/kg of SOIL)

Compound	Texas Soil		Mississippi Soil	
	Acceptable+	Actual	Acceptable*	Actual
Acid Extractables				
Phenol	720	700	320	350
o-Cresol	490	500	250	250
p-Cresol	100	100	50	45
m-Cresol	124	120	130	130
2-Chlorophenol	380	400	290	300
3-Chlorophenol	120	120	60	55
4-Chlorophenol	88	90	45	50
2,3-Dichlorophenol	130	130	60	60
2,4-Dichlorophenol	90	90	45	40
2,5-Dichlorophenol	300	300	30	30
2,6-Dichlorophenol	630	630	50	48
3,4-Dichlorophenol	30	30	23	22
2,4,5-Trichlorophenol	40	40	30	30
2,4,6-Trichlorophenol	300	300	215	220
Pentachlorophenol	30	30	55	50
2,4-Dimethylphenol	88	90	45	40
2-Methyl-4-Chlorophenol	48	50	38	40
3-Methyl-4-Chlorophenol	18	20	10	10
3-Methyl-6-Chlorophenol	150	150	140	140
p-Nitrophenol	250	250	45	50
2,4-Dinitrophenol	1380	140	270	330
4,6-Dinitro-o-Cresol	350	300	26	105
Amines				
Toluenediamine	1080	500	1400	680
Brucine	3600	180	3200	150
Alcohols				
Isobutyl alcohol	++	925	++	940
Allyl alcohol	++	750	++	700
Propargyl alcohol	8200	980	5100	930
1-Butanol	++	870	++	850
2,3-Dichloropropanol	1050	1200	700	700
Methanol	++	740	++	740
Other				
2-Nitropropane	1620	640	1170	540
Thiourea	6580	660	910	100

+ *As determined by the procedure described in Section 4, data from Table 10*

* *As determined by the procedure described in Section 4, data from Table 11*

++ *See text, Section 4: acceptable loading rate was considerably greater than 1000 mg/kg.*

TABLE 18. LOSS RATES, CORRELATION COEFFICIENTS AND 95% CONFIDENCE INTERVALS FOR SPECIFIC CHEMICALS -- TEXAS SOIL

COMPOUND	KINETIC PARAMETERS					
	FIRST ORDER			ZERO ORDER		
	HALF LIFE (day)	r*	95% C.I.	mg/Kg/day	r*	95% C.I
Acid Extractables						
Phenol	4.1	0.92	3.1-6.1	59.3	0.96	46.9-71.4
o-Cresol	1.6	0.83	1.5-1.7	62.2	0.95	48.5-75.9
p-Cresol	(1)	--	--	>100 (1)	--	--
m-Cresol	0.6	0.87	0.4-1.4	28.2	0.96	20.5-36.9
2-Chlorophenol	1.7	0.98	1.5-1.9	26.2	0.90	20.4-32.0
3-Chlorophenol	21.8	0.97	19.6-24.6	2.3	0.95	2.0-2.6
4-Chlorophenol	1.0	0.91	0.7-1.3	14.5	0.91	9.7-19.2
2,3-Dichlorophenol	8.3	0.97	7.4-9.3	3.6	0.98	3.2-3.9
2,4-Dichlorophenol	1.5	0.94	1.1-1.9	14.1	1.00	13.0-15.2
2,5-Dichlorophenol	16.6	0.99	15.6-17.7	6.1	0.96	5.4-6.8
2,6-Dichlorophenol	2.4	0.91	2.0-3.0	41.8	0.97	36.7-46.9
3,4-Dichlorophenol	3.2	0.89	2.3-4.9	2.7	0.95	2.1-3.3
2,4,5-Trichlorophenol	14.6	0.95	12.2-18.1	1.4	0.96	1.2-1.6
2,4,6-Trichlorophenol	5.3	0.94	4.6-6.3	10.4	0.94	8.8-12.1
Pentachlorophenol	6.7	0.92	5.5-8.5	1.0	0.91	0.8-1.2
2,4-Dimethylphenol	0.7	0.99	0.6-0.8	27.3	0.90	18.1-36.5
2-Methyl,4-Chlorophenol	2.9	0.90	2.7-3.0	3.7	0.94	3.0-4.4
3-Methyl-4-Chlorophenol	1.4	0.99	1.2-1.6	3.8	0.93	2.1-5.4
3-Methyl, 6-Chlorophenol	2.1	0.98	1.9-2.4	11.2	0.94	9.2-13.1
p-Nitrophenol	10.2	0.81	7.5-16.3	6.8	0.92	5.3-8.3
2,4-Dinitrophenol	4.6	0.92	2.9-11.7	12.9	0.96	7.5-18.3
4,6-Dintro-o-Cresol	(2)	--	--	(2)	--	--
Amines						
Toluenediamine	11.9	0.65	7.5-28.5	5.9	0.47	0.7-11.2
Brucine	23.1	0.86	18.0-33.0	3.1	0.81	2.4-4.2
Alcohols						
Isobutyl Alcohol	2.4	0.96	2.1-2.9	172	0.96	144-200
Allyl Alcohol	10.2	0.95	8.8-12.2	30.0	0.93	24.3-35.6
Propargyl Alcohol	12.6	0.94	10.7-15.2	29.5	0.97	26.2-32.9
1-Butanol	1.0	0.75	0.7-2.4	184	0.90	130-239
2,3-Dichloropropanol	23.1	0.97	20.9-26.0	22.2	0.94	18.7-25.6
Methanol	1.0	0.75	0.68-2.4	184	0.94	140-228
Other						
2-Nitropropane	0.5	0.97	0.4-0.6	155	0.77	79-230
Thiourea	12.8	0.86	9.9-18.0	2.1	0.95	1.8-2.4

(1) *No chemical was detected after one day.*

(2) *No loss during the 65-day experiment*

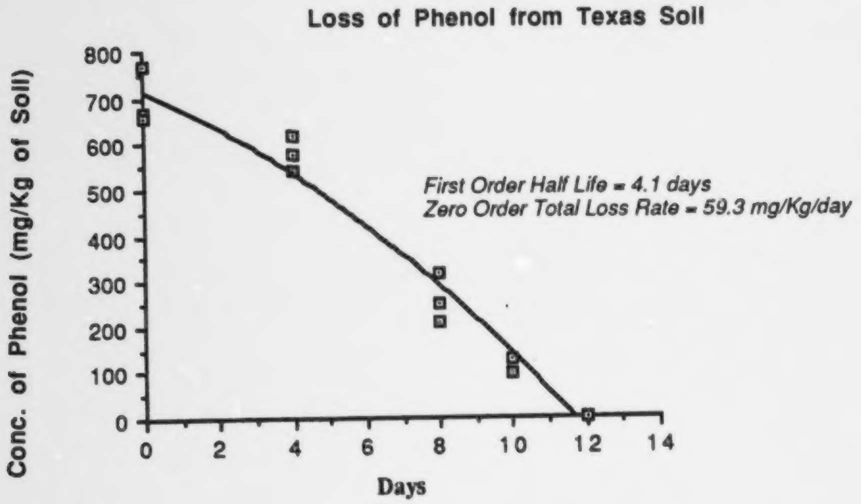
* *Correlation coefficient*

TABLE 19. LOSS RATES, CORRELATION COEFFICIENTS AND 95% CONFIDENCE INTERVALS FOR SPECIFIC COMPOUNDS -- MISSISSIPPI SOIL

COMPOUND	KINETIC PARAMETERS					
	FIRST ORDER			ZERO ORDER		
	HALF LIFE (day)	r*	95% C.I.	mg/Kg/day	r*	95% C.I
Acid Extractables						
Phenol	23.0	0.95	19.6-27.8	7.0	0.93	5.6-8.4
o-Cresol	5.1	0.88	4.0-6.7	6.4	0.97	5.7-7.1
p-Cresol	0.5	0.93	0.4-0.8	23.2	0.88	11.8-34.5
m-Cresol	11.3	0.97	9.7-13.4	5.0	0.96	4.1-5.9
2-Chlorophenol	7.2	0.95	6.3-8.7	8.4	0.88	6.2-10.6
3-Chlorophenol	15.1	0.97	13.6-17.1	1.3	0.95	1.1-1.5
4-Chlorophenol	2.5	0.96	2.1-3.1	5.6	0.90	3.6-7.5
2,3-Dichlorophenol	18.3	0.84	13.9-26.8	1.0	0.79	0.6-1.3
2,4-Dichlorophenol	3.5	0.95	2.9-4.6	2.3	0.85	1.3-3.3
2,5-Dichlorophenol	18.5	0.94	16-22	5.2	0.87	3.9-6.5
2,6-Dichlorophenol	16.2	0.94	13.9-19.5	8.9	0.88	6.5-10.6
3,4-Dichlorophenol	18.3	0.90	15.0-23.4	0.5	0.91	0.4-0.6
2,4,5-Trichlorophenol	22.3	0.95	19.1-26.9	0.6	0.92	0.5-0.7
2,4,6-Trichlorophenol	6.3	0.93	5.3-7.8	5.7	0.94	4.7-6.7
Pentachlorophenol	12.0	0.94	10.2-14.6	1.1	0.94	0.9-1.3
2,4-Dimethylphenol	1.4	0.99	1.2-1.6	6.7	0.96	4.9-8.5
2-Methyl,4-Chlorophenol	6.3	0.94	5.2-7.7	1.8	0.87	1.2-2.3
3-Methyl-4-Chlorophenol	4.2	0.92	3.2-5.9	0.9	0.88	0.5-1.2
3-Methyl, 6-Chlorophenol	12.5	0.93	10.6-15.3	3.0	0.85	2.1-3.9
p-Nitrophenol	2.5	0.81	1.4-5.8	3.6	0.93	1.9-5.3
2,4-Dinitrophenol	32.1	0.96	25.9-42.1	3.7	0.89	1.5-5.5
4,6-Dintro-o-Cresol	(1)	--	--	(1)	--	--
Amines						
Toluenediamine	6.5	0.85	4.9-9.7	12.0	0.57	2.7-21.2
Brucine	37.1	0.66	21.7-126.3	2.7	0.59	0.4-4.9
Alcohols						
Isobutyl Alcohol	11.3	0.66	7.7-20.8	37.7	0.73	23.1-52.3
Allyl Alcohol	9.5	0.93	7.9-11.8	26.9	0.96	23.0-30.8
Propargyl Alcohol	13.0	0.89	10.6-16.6	23.7	0.96	20.6-26.7
1-Butanol	8.5	0.60	5.3-21.2	39.6	0.79	25.9-53.3
2,3-Dichloropropanol	55.3	0.92	45.8-69.5	10.9	0.91	8.5-13.4
Methanol	3.2	0.73	2.3-5.6	68	0.96	59-76
Other						
2-Nitropropane	0.66	0.95	0.56-0.79	115	0.84	76-154
Thiourea	18.7	0.45	-8.3 to +76.2	2.7	0.50	-0.22 to +5.6

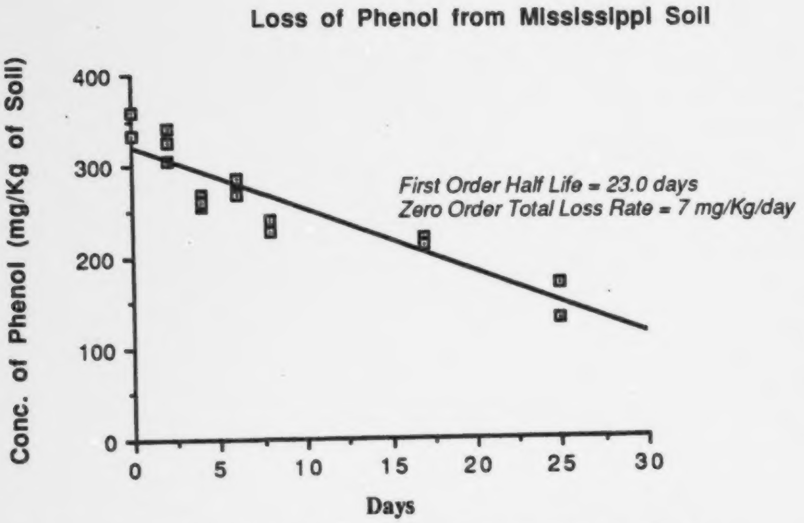
(1) *No loss during the 65-day experiment*

* *Correlation coefficient*



First Order Half Life = 4.1 days
Zero Order Total Loss Rate = 59.3 mg/Kg/day

Figure 2. Loss of Phenol in Texas Soil at 20° C.
Moisture content was maintained between 12 and 16%.
The initial chemical loading was 700 mg/kg.



First Order Half Life = 23.0 days
Zero Order Total Loss Rate = 7 mg/Kg/day

Figure 3. Loss of Phenol in Mississippi Soil at 20° C.
Moisture content was maintained between 12 and 16%.
The initial chemical loading was 350 mg/kg.

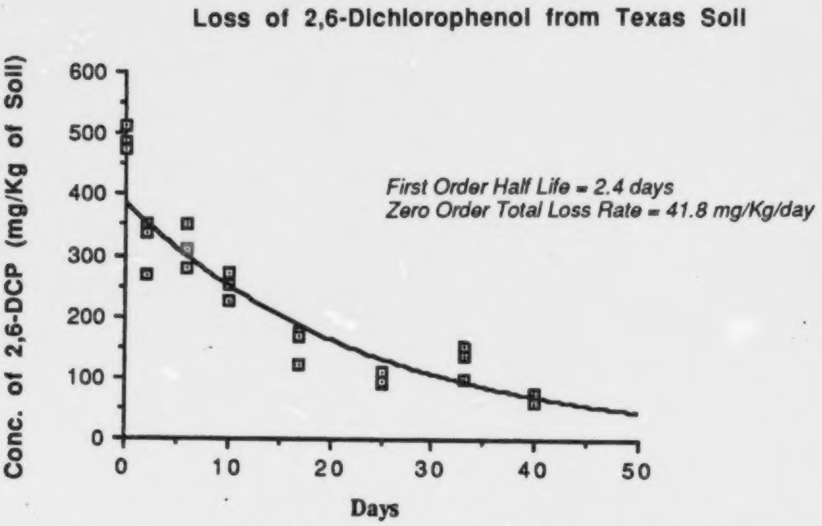


Figure 4. **Loss of 2,6-Dichlorophenol in Texas Soil at 20° C.** Moisture content was maintained between 12 and 16%. The initial chemical loading was 630 mg/kg.

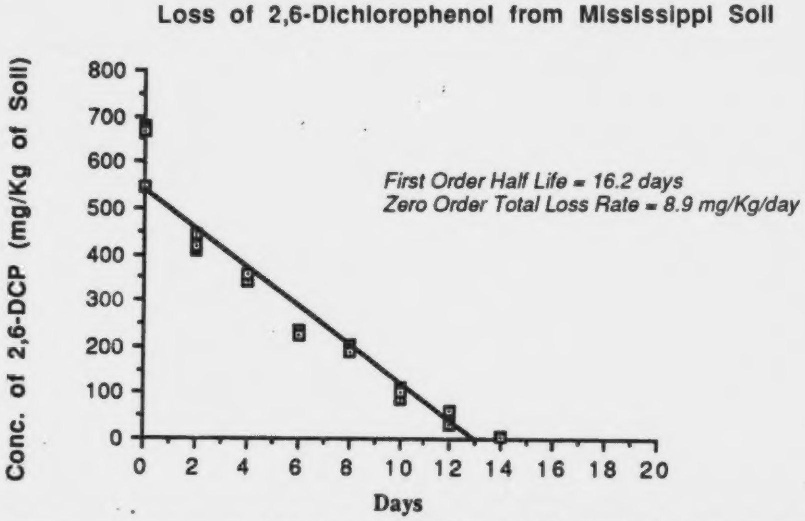


Figure 5. **Loss of 2,6-Dichlorophenol in Mississippi Soil at 20° C.** Moisture content was maintained between 12 and 16%. The initial chemical loading was 48 mg/kg.

it was not possible to discern whether zero or first order kinetics were a better representation of the data.

Generally the rates of chemical loss were higher in the Texas soil than the Mississippi soil (Table 20). There were a few situations in which the chemical loss rates were about the same or greater in the Mississippi soil than those in the Texas soil. There did not appear to be any particular pattern to the differences of rates in the two soils. Because the study was not designed to determine the reasons that such differences occur, it was not possible to identify the factors causing the differences. However, there are several possibilities. These include: (a) different sorption characteristics and solubilities and therefore different availability of the chemical for microbial degradation, and (b) the different pH in the soils and the effect of pH on chemical dissociation and the form of the chemical available for degradation. Some discussion of these possibilities was presented in Section 4.

Care should be taken in extrapolating these chemical loss rates to field conditions, since such conditions can be different than those in the laboratory microcosms. For example, these loss rates were obtained at 20° C, with no nutrient additions, with a reasonable but narrow range of moisture, without pH control, without acclimated microorganisms, under quiescent conditions, and each chemical was evaluated separately. At field sites, moisture and temperature can vary considerably over the seasons, nutrients may be added to assure microbial growth, and soils may be limed. Typically, chemicals are added as mixtures and acclimated microorganisms will exist at the site after repeated waste applications. In addition higher chemical loading rates can occur. Thus, in attempting to utilize these loss rates to predict chemical loss at specific sites, the differences between laboratory and field conditions should be recognized and taken into account.

In Section 4, the data indicated that the substitution position of a chemical on the phenol ring and the type of chemical group that was substituted affected the

TABLE 20. CHEMICAL HALF-LIVES IN TEXAS AND MISSISSIPPI SOILS (days)

Compound	Texas Soil	Mississippi Soil
Acid Extractables		
Phenol	4.1	23.0
o-Cresol	1.6	5.1
p-Cresol	(1)	0.5
m-Cresol	0.6	11.3
2-Chlorophenol	1.7	7.2
3-Chlorophenol	21.8	15.1
4-Chlorophenol	1.0	2.5
2,3-Dichlorophenol	8.3	18.3
2,4-Dichlorophenol	1.5	3.5
2,5-Dichlorophenol	16.6	18
2,6-Dichlorophenol	2.4	16.2
3,4-Dichlorophenol	3.2	18.3
2,4,5-Trichlorophenol	14.6	22.3
2,4,6-Trichlorophenol	5.3	6.3
Pentachlorophenol	6.7	12.0
2,4-Dimethylphenol	0.7	1.4
2-Methyl-4-Chlorophenol	2.9	6.3
3-Methyl-4-Chlorophenol	1.4	4.2
3-Methyl-6-Chlorophenol	2.1	12.5
p-Nitrophenol	10.2	2.5
2,4-Dinitrophenol	4.6	32.1
Amines		
Toluenediamine	11.9	6.5
Brucine	23.1	37.1
Alcohols		
Isobutyl alcohol	2.4	11.3
Allyl alcohol	10.2	9.5
Propargyl alcohol	12.6	13.0
1-Butanol	1.0	8.5
2,3-Dichloropropanol	23.1	55.3
Methanol	1.0	3.2
Other		
2-Nitropropane	0.5	0.66
Thiourea	12.8	18.7

(1) *No chemical was detected after one day.*

relative toxicity and the acceptable loading rate data. Each chemical group and substitution position also was evaluated to see their effect on the degradation rate data.

When the half-life data are compared for the fourteen chlorinated phenols with chlorine in different positions on the phenol ring (Table 21), it appeared that the compounds with the chlorine substituted in the meta position had the greater half-lives and therefore the lowest loss rate. This was particularly evident with the mono-, di-, and trichlorophenols. Using the data in Table 21, it was not possible to discern whether there were any real differences when the chlorine was in the ortho and para positions. These results are somewhat different from those in Section 4 where relative toxicity of these compounds appeared related to the substitution position of the chlorine group with the order being para>meta>ortho.

When the data from non-chlorinated phenols were compared (Table 22), all of the methylphenols were lost very rapidly in the Texas soil. In the Mississippi soil, the chemical loss rate was considerably slower when the methyl group was substituted in the meta position.

In Table 23, data for chemicals with different compounds substituted on the phenol ring were compared. The data suggested that compounds that had a nitro compound substitution on the phenol ring had a lower loss rate. No other differences in terms of type of compound substitution were apparent.

CONCLUSIONS

1. The chemical or waste loading procedure (Table 9, Section 4) resulted in chemical loadings that did not inhibit the non-acclimated organisms in the laboratory microcosms, except in one case (4,6-Dinitro-o-Cresol). Thus, this procedure provided a good estimate of initial, acceptable chemical loadings that can be used in laboratory degradation studies.

TABLE 21. EFFECT OF SUBSTITUTION POSITION ON DEGRADATION RATES -- CHLORINATED PHENOLS

Compound	Chemical Half-Life (days)		Substitution Position
	Texas Soil	Mississippi Soil	
2-Chlorophenol	1.7	7.2	ortho
3-Chlorophenol	21.8	15.1	meta
4-Chlorophenol	1.0	2.5	para
2,3-Dichlorophenol	8.3	18.3	ortho, meta
2,4-Dichlorophenol	1.5	3.5	ortho, para
2,5-Dichlorophenol	16.6	18.5	ortho, meta
2,6-Dichlorophenol	2.4	16.2	ortho, ortho
3,4-Dichlorophenol	3.2	18.3	meta, para
2,4,5-Trichlorophenol	14.6	22.3	ortho, para, meta
2,4,6-Trichlorophenol	5.3	6.3	ortho, para, ortho
Pentachlorophenol	6.7	12.0	all
2-Methyl-4-Chlorophenol	2.9	6.3	ortho, para
3-Methyl-4-Chlorophenol	1.4	4.2	meta, para
3-Methyl-6-Chlorophenol	2.1	12.5	meta, ortho

TABLE 22. EFFECT OF SUBSTITUTION POSITION ON DEGRADATION RATES -- NON-CHLORINATED PHENOLS

Compound	Chemical Half-Life (days)		Substitution Position
	Texas Soil	Mississippi Soil	
Methylphenols			
o-Cresol (2-Methylphenol)	1.6	5.1	ortho
p-Cresol (4-Methylphenol)	(1)	0.5	para
m-Cresol (3-Methylphenol)	0.6	11.3	meta
2,4-Dimethylphenol	0.7	1.4	ortho, para
Nitrophenols			
p-Nitrophenol	10.2	2.5	para
2,4-Dinitrophenol	4.6	32.1	ortho, para

(1) *No chemical was detected after one day.*

TABLE 23. COMPARATIVE DEGRADATION RATES OF CHLORO-, METHYL- AND NITROPHENOLS

Compound	Chemical Half-Life (days)	
	Texas Soil	Mississippi Soil
3-Chlorophenol	21.8	15.1
m-Cresol (3-Methylphenol)	0.6	11.3
p-Nitrophenol	10.2	2.5
p-Cresol (4-Methylphenol)	(1)	0.5
4-Chlorophenol	1.0	2.5
2,4-Dinitrophenol	4.6	32.1
2,4-Dimethylphenol	0.7	1.4
2,4-Dichlorophenol	1.5	3.5

(1) *No chemical was detected after one day.*

2. Based on the data, it was not possible to discern whether zero or first order kinetics provided a better representation of data. For most of the chemicals the data could be fit to either kinetics with high correlation coefficients.
3. In general, the rates of chemical loss were higher in the Texas soil than in the Mississippi soil. There did not appear to be any pattern to the differences in rates in the two soils.
4. Chlorophenols with chlorine substituted in the meta position had greater half-lives and therefore lower chemical loss rates. This was particularly evident with the mono-, di- and trichlorophenols in the Texas soils.
5. Chemicals that had a nitro group substituted on the phenol ring appeared to have a lower loss rate.

SECTION 6

ADSORPTION EXPERIMENTS

INTRODUCTION

The persistence of hazardous organic compounds in soils is related to reactions that affect the transport and fate of such chemicals. One of the most important reactions is adsorption.

Adsorption is the process by which ions or molecules present in one phase tend to concentrate at a surface or interface. The process can occur at an interface betweenl any two phases, such as liquid-liquid, gas-liquid, or solid-liquid interfaces. The adsorbed substance is the adsorbate while the adsorptgion phase is referred to as the adsorbent. The tendency of organic molecules to adsorb on soil is determined by the physical and chemical characteristics of the chemical compound and the soil to which it is added. The two driving forces for adsorption are the lyophobic (solvent-disliking) character of a solute relative to a particular solvent, and the affinity of the solute for the solid, such as electrical attraction or van der Waal's attraction(19).

Adsorption is the major retention mechanism for most organic and inorganic compounds in soils. As a result, the leaching potential of a chemical in soil is, in general, proportional to the magnitude of the adsorption (partitioning) coefficient of that chemical in a soil. The adsorption potential of a chemical is governed by the properties of both the soil and the chemical. Important properties of the chemical that affect adsorption include: (a) chemical structure, (b) acidity or basicity of the molecule (pK_a or pK_b), (c) water solubility, (d) permanent charge, (e) polarity, and (f) molecule size.

To estimate the environmental movement of a chemical, values of the adsorption coefficient and other sorption equation coefficients can be compared to the value of other chemicals whose behavior in soil and sediment systems is well documented.

Adsorption Equilibria

At equilibrium, the solute remaining in solution is in dynamic equilibrium with that of the soil surface. At this point, there is a defined distribution of solute between the liquid and solid phases. The preferred form for depicting this distribution is to express the quantity q_e (amount of solute sorbed per unit weight of solid sorbent) as a function of the equilibrium solution concentration (C_e) at a fixed temperature. An expression of this type is an adsorption isotherm.

One of the oldest adsorption equations that has been used widely for solid-liquid systems is the Freundlich equation:

$$q_e = K_f C_e^{1/n} \tag{6}$$

where q_e is the equilibrium distribution coefficient (mg of chemical/gm of adsorbent), C_e is the equilibrium chemical concentration (mg/liter of solvent), and K_f and 1/n are constants. The constant, K_f, is related to the capacity or affinity of the adsorbent and the exponential term, 1/n, is an indicator of the intensity, or how the capacity of the adsorbent varies with the equilibrium solute concentration. The Freundlich isotherm has had success in describing sorption behavior of organics(19) and the adsorption data generated in this study were compared to this empirical model.

The Freundlich constants may be determined statistically when the equation is expressed in linear form by a logarithmic transformation:

$$\log q_e = \log K_f + 1/n \log C_e \tag{7}$$

The constants, K_f and 1/n, can be obtained, respectively, from the intercept and slope of log-log plots of q_e vs C_e.

The Freundlich equation that results from specific experiments should not be extrapolated beyond the experimental range of data used in its construction. This is because the Freundlich equation predicts infinite adsorption at infinite concentrations, and that any soil or clay has an unlimited capacity to retain chemicals dissolved in water. Such an infinite capacity is not only thermodynamically inconsistent, but experience has shown that the extent of adsorption ultimately is limited by the surface area of the adsorbent. Thus, the Freundlich equation cannot be extrapolated with confidence beyond the experimental range used in its construction and will not yield a maximum capacity term. The latter term is a convenient single-valued number that estimates the maximum amount of adsorption beyond which the soil surfaces are saturated and no further net adsorption can be expected.

Soil Organic Carbon

Sorption of nonionic organic compounds from water onto soil has been shown(20) to occur primarily by partition onto the soil organic phase. Adsorption by soil minerals is relatively unimportant in wet soils, presumably because of strong dipole interaction between the soil minerals and water, which excludes neutral organic solutes from this portion of soil. Therefore, the more organic matter in soil, the more adsorption is expected. Soil organic matter has been the single best predictor of the adsorption isotherm parameter(21-23), and the use of the soil organic matter-water partition coefficient, K_{om}, rather than the adsorption partition coefficient, K_D, has been proposed as more appropriate:

$$K_{om} = q_{om}/C \tag{8}$$

where q_{om} = mg adsorbed/g soil organic matter and C = liquid phase concentration, mg/L.

Organic matter content can be obtained by measurement or by multiplying an experimentally determined organic carbon concentration by an appropriate conversion factor. Because various researchers used different conversion factors, the soil organic carbon content has been proposed(20, 23) to normalize adsorption partition coefficients. This parameter (K_{oc}) is:

$$K_{OC} = q_{OC}/C = (K_D/\%OC)\ 100 \tag{9}$$

where q_{OC} = mg sorbed/g soil organic carbon, %OC = (mass of organic carbon/mass of soil) 100, %OM = %OC (f), and f = conversion factor ranging from 1.7 to 2.0.

Rao et al.(24) performed an exhaustive literature search, showed that coefficient of variation (CV) values for K_{OC} were much lower than those of K_D or K (Freundlich coefficient), and suggested that K_{OC} be used as a universal adsorption partition coefficient. This relationship is valid when the organic carbon content of the soil is more than 0.1%.

With regard to inorganics, the sorptive effect of the inorganic matrix was indicated to be negligible at an organic content level of 1% and above(25). However, for some sorbents, chemical interaction with the inorganic matrix may be important. In the absence of organic carbon, the specific surface area and the nature of the mineral surface have a greater impact on the degree of sorption(26).

Soil pH

The pH of a soil-water system can affect the sorption of organic solutes. Because the extent of ionization of an acidic or basic compound affects its adsorption, pH affects adsorption in that it governs the degree of ionization(19). Except with ion exchange adsorption, ions tend to be less readily adsorbed than neutral species. Many organics form negative ions at high pH, positive ions at low pH, and neutral species in intermediate pH ranges. Generally adsorption is increased at pH ranges where the species are neutral in charge. pH also affects the charge on the soil surface, altering its ability to adsorb materials(27).

In general, adsorption of organic pollutants from water increases with decreasing pH. In many cases, this may result from neutralization of negative charges at the soil surface with increasing hydrogen ion concentration, thereby reducing hindrance to diffusion and making more available the active surface of the species(19). Usually, organic acids are more adsorbable at low pH, whereas the adsorption of organic bases is favored by high pH. The optimum pH for any adsorption process must be determined by laboratory testing.

Sorption of the non-dissociated chemical species and also of their anionic species can occur(28). When the pK_a of a species, such as pentachlorophenol, is relatively low compared to a natural water system, anionic species adsorption can occur. For highly chlorinated phenols, prediction of overall distribution ratios on simple partitioning of the nondissociated species can be in error(28).

Another soil adsorption characteristic which is influenced by soil pH is the Cation Exchange Capacity (CEC). At very low pH values, only a small portion of the positive ions held by the clays and organic colloids can be replaced by cation exchange. As pH increases, the hydrogen held by organic colloids and silicate clays becomes ionized and can be exchanged by positively charged organic molecules.

Ion exchange is hypothesized to dominate the sorption process in acidic soil(29). The higher sorption in the acidic soil, as compared to basic soil, reflects stronger sorption of the protonated organic cations. Competitive adsorption occurs between compounds in an acidic soil where the protonated compound species predominates in solution. In contrast, competition is minimal in a basic soil when the compounds are neutral. Non-ionic organic compounds may sorb independently on soil from a mixture(20).

When the protonated and neutral species coexist, site-specific sorption of the cation is preferred because of the electrostatic attraction between the base and the negatively charged soil surface(29). When anionic and neutral species coexist, neutral species sorption occurs because of the electrostatic repulsive forces of the anionic species(30). Maximum adsorption is attained near the point where $pH = pK_a$, and sorption capacity drops rapidly at pH values above pK_a.

MATERIALS AND METHODS

Adsorption Method

The adsorption of the specific chemicals was investigated in batch adsorption experiments. A list of the chemicals investigated and a description of the soil types used were presented earlier (Tables 1 and 3). The batch adsorption technique(31) consists of mixing an aqueous solution containing a solute of known composition and concentration with a given mass of sorbent (soil) for a period of time. The solution is then separated from the sorbent and chemically analyzed to determine changes in solute concentration. The amount of solute sorbed is assumed to be the difference between the initial concentration (before contact with the sorbent) and the solute concentration after mixing.

Stock Solutions

The solvent used was distilled deionized water (DDW) obtained by passage through a Barnstead Water Purification Cartridge #DO 809. The stock solution of each organic compound contained the maximum soluble amount of the compound in DDW at room temperature (25°C). The procedure used to prepare the stock solutions and to determine the solubility limit of specific chemicals is noted in Table 24.

The solubility limits of chemicals determined as part of this study are included in Table 25. This table contains only those chemicals whose maximum aqueous solubilities were determined as part of this research. The solubilities of chemicals not listed in Table 25 were obtained from the literature(32).

Standard Solution

To prepare a calibration curve for a chemical, an accurately prepared standard solution is required. The following procedure was used to prepare the standard solution of each compound. A calculated amount of the compound and 100 mL of DDW was added to an oven-dried 100 mL volumetric flask. The solution was mixed

TABLE 24. PROCEDURE FOR DETERMINING SOLUBILITY LIMIT OF AN ORGANIC COMPOUND IN WATER

(a) Put distilled deionized water into a teflon-capped bottle (4 L).

(b) Add enough organic compound to the bottle to observe some nonsoluble (liquid) or particulate (solid) organic compound in the water phase.

(c) Stir the solution at room temperature (20° C) with magnetic stirrer.

(d) Add enough compound to produce a saturated solution. This will be identified when there is residual insoluble compound remaining in the bottle after the mixing period.

(e) Before the filtration, prewash a filter with about 100 mL of water, then rinse the prewashed filter with about 10 mL of the saturated solution. The prewashing procedure can remove any so-called wetting agent from the filter .

(f) Filter solution with 0.45µm pore size membrane filter.

(f) Determine the concentration of organic compound in the filtrate by HPLC.

(g) The HPLC result is the solubility limit in water. The filtrate is transferred into a teflon-capped amber bottle and stored at 4° C room. This is the stock solution for adsorption test. Prior to use, this solution is mixed at 20° C.

TABLE 25. LIST OF MAXIMUM SOLUBILITIES (20° C) IN WATER DETERMINED AS PART OF THE ADSORPTION STUDIES

Compound	Concentration (mg/L)
Acid Extractables	
2-Chlorophenol	20,300
3-Chlorophenol	17,000
4-Chlorophenol	8,650
2,3-Dichlorophenol	3,900
2,4-Dichlorophenol	2,600
2,5-Dichlorophenol	3,600
2,6-Dichlorophenol	1,500
3,4-Dichlorophenol	520
2,4,5-Trichlorophenol	930
2,4,6-Trichlorophenol	415
Pentachlorophenol	13
2,4-Dimethylphenol	7,500
2-Methyl-4-Chlorophenol	2,500
3-Methyl-4-Chlorophenol	3,900
3-Methyl-6-Chlorophenol	1,400
p-Nitrophenol	5,300
2,4-Dinitrophenol	500

Compound	Concentration (mg/L)
Amines	
Diphenylamine	2.5
Toluenediamine	590
Brucine	310
Alcohols	
Isobutyl alcohol	3,700
Allyl alcohol	4,300
Propargyl alcohol	4,900
1-Butanol	4,200
2,3-Dichloropropanol	7,600
Other	
2-Nitropropane	2,800
Thiourea	5,000
Explosive Chemicals	
2,4-Dinitrotoluene	160
2,4,6-Dinitrotoluene	610
RDX	42
HMX	3.8

well until no particulate matter was visible. This known concentration was diluted to five concentrations ranging from 10 mg/L to 100 mg/L (usually 10, 30, 50, 70 and 100 mg/L) since five data points are needed to construct the calibration curve. The five standard solutions for each compound were stored at 4° C and used whenever soil extracts containing the compound were analyzed by HPLC.

Soil Moisture

The soil moisture content is needed to calculate the amount of chemical sorbed per unit of dry soil. The standard ASTM procedure(33) for measuring soil moisture content was followed (Table 26).

Soil:Solution Ratio

A single soil:solution ratio may not be satisfactory for all organic compounds. This is because a weakly adsorbed compound may not result in a measurable concentration change after contact with soil. Conversely, a compound's affinity for soil may be so strong that the final solution concentration is below analytical detection limits.

TABLE 26. PROCEDURE FOR MEASURING SOIL MOISTURE CONTENT (Taken from ASTM D2216)(33)

(a) Place aluminum pans in an 105° oven for 24 hours. Transfer them to a dessicator at room temperature (20° C) for approximately 1 hour. Measure and record the weight of an aluminum pan on the analytical balance (w_1 grams). Precision of the fourth decimal place is required.

(b) Weigh out approximately thirty grams of the sorted soil (w_2 grams) into dried aluminum pans.

(c) Dry soil at 105° C for 48 hours.

(d) Measure and record the weight of the dried soil and the aluminum pan (w_3 grams).

(e) Calculate soil moisture content, W (%) using the following equation:

$$W = \frac{\text{weight of moisture}}{\text{weight of oven-dried soil}} \times 100 = \frac{w_2 - w_3}{w_3 - w_1} \times 100$$

To determine an optimum soil:solution ratio for each compound, batch sorption tests were performed using several soil:solution ratios. To evaluate the optimum soil:solution ratio, the adsorption characteristics of the compounds at two different initial compound concentrations were evaluated. The maximum concentration used for the adsorption measurements was the solubility limit of the compound in water. The minimum concentration used was about one order of magnitude higher than the lowest detection limit determined by high pressure liquid chromatography. The procedure for determining the optimum soil:solution ratio is outlined in Table 27.

TABLE 27. PROCEDURE FOR DETERMINATION OF OPTIMUM SOIL:SOLUTION RATIO

(a) Set soil-to-solution ratio at 1:1 to 1:X. Required mass of the sorted soil, Ms (gram), and volume of solution, V (mL), are calculated by:

$$\frac{M_s\,(1-(W/100))}{V + M_s\,(W/100)} = \frac{1}{X}$$

where W = moisture content of the soil.

(b) Weigh out the desired amount of sorted soil (M_s grams) into an amber bottle.

(c) Add V mL of solution to the amber bottle. This is designated as a sample. Prepare another bottle which will not contain any soil. This is designated as the blank.

(d) Place bottles in rotating tumbler in the 20° C room and tumble continuously for 24 hours at 30 rpm.

(e) After tumbling, centrifuge the bottles for 30 minutes at 4,000 rpm.

(f) After centrifugation, filter the supernatant with a glass fiber filter and then with a 0.45 μm pore size membrane filter.

(g) Analyze the concentrations of the filtrate by HPLC.

(h) Calculate the percent absorption P(%):

$$P(\%) = \frac{C_{fb} - C_{fs}}{C_{fb}} \times 100$$

where C_{fb} = final concentration of blank (mg/L), and
C_{fs} = final concentration of sample (mg/L).

(j) Use another soil-to-solution ratio, and repeat (a) through (h).

(k) The optimum soil solution ratio is one that satisfies the condition that P(%) is between 20% and 80%.

Solute Stability

In conducting batch adsorption experiments, it is important to consider the stability of the solute in solution. Processes such as photolysis, hydrolysis, and microbial degradation can cause a decrease in solute concentration leading to errors in adsorption results.

In this study, photolysis was minimized by using amber bottles to limit transmission of light during solution/soil mixing. By limiting contact time to one day and using an unacclimated soil, chemical losses due to chemical and microbial degradation were minimal. Chemical losses due to hydrolysis were not quantified and thus are included in adsorption calculations.

Other Factors

Other factors which affect adsorption efficiency include temperature and ionic strength. The sorption experiments were conducted at 20° C. All experiments were conducted at the pH of the soil:solution which was approximately that of the respective soils. No pH adjustment of the soil solution was made.

Adsorption is not instantaneous and mixing for a specific amount of time is necessary to assure equilibrium. The equilibration time should be the minimum time beyond which relatively insignificant changes in the solute concentration will occur. In this study, a separate set of experiments was conducted to determine the effect of mixing time on the amount of chemical adsorbed. The results indicated that the amount of adsorption, q (mg sorbed/g soil) slightly increased with time. However, the q values did not vary greatly with time after 24 hours. The data representing the amount of chemical adsorbed after 24 hours were analyzed statistically. The slope of q with time was not statistically different from zero and q was shown not to vary with time after 24 hours. This implied that 24 hours of mixing was enough for equilibrium, and thus mixing for one day was used in the batch adsorption studies.

Data Analysis

At least four data points are needed to construct the Freundlich isotherms and to determine the appropriate coefficients. In this study, a minimum of four and frequently as many as eight data points were used to develop the isotherms. The isotherm data were fit to the Freundlich adsorption equation (Eqn. 6). A log-log scale was used to plot the Freundlich isotherm. The abscissa was the equilibrium (final) concentration in liquid phase and the ordinate was the amount adsorbed per unit of soil.

The data were analyzed by least-square linear regression methods with 95% confidence interval (CI) determined as described in Section 4. Routine statistical methods were used(18). First, the linear relation between X_i (= log C_i) and Y_i (= log q_i) was determined by the Lotus 1-2-3 program:

$$Y = a + b X \tag{7}$$

where a = intercept and b = slope of the line.

Second, the variances of a and b, S_a^2 and S_b^2 were calculated, respectively by:

$$S_a^2 = S_y^2 \ \{1/n + X_{avg}^2/(\Sigma X_i - X_{avg})^2\} \tag{8}$$

$$S_b^2 = S_y^2/(\Sigma X_i - X_{avg})^2 \tag{9}$$

where

$S_y^2 = \{\Sigma(Y_i - Y_{lin,\,i})^2/(n-2)\}^{0.5}$,
X_i, Y_i = individual data point of log C_i, log q_i, respectively,
X_{avg} = average of X_i,
$Y_{lin,i}$ = estimated Y_i value with respect to X_i from the linear relationship $Y = a+b X$, and
n = number of samples.

The 95% CI for the estimates of a and b were determined by a-t S_a to a+t S_a and b-t S_b to b+t S_b, respectively, where the t value can be found in a typical statistical text for a two-sided test with a degree of freedom = n-2, and alpha = 5% level of significance.

RESULTS

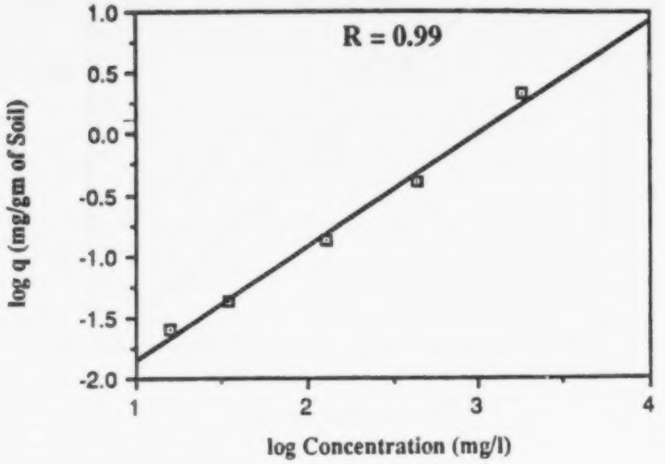
Following the procedures described above, the adsorption isotherms and constants were determined. Illustrative sorption patterns and isotherms are noted in Figures 6 and 7. The isotherm data are presented in Tables 28 and 29. Based on the correlation coefficients calculated for the data, the Freundlich equation described the adsorption satisfactorily for all but a few chemicals in both soils.

As described earlier, the pH of the solution can affect the sorption characteristics. In this study, no attempt was made to control the pH of the extracts to any set value. Therefore, the pH of the extracts varied between soil types and between samples of the same soil type (Tables 28 and 29). In the Texas soil, the pH range varied from 7.5 to 8.0 while in the Mississippi soil it ranged from 4.5 to 7.0. In evaluating and using the sorption data, the different pH ranges should be recognized.

As noted earlier, the adsorption coefficients represent the results obtained over a specific chemical concentration range and the Freundlich equation that results for a chemical should not be extrapolated beyond that range. Tables 30 and 31 indicate the chemical concentration ranges for which the data in Tables 28 and 29 are valid.

A wide range of chemical concentrations was evaluated, i.e., from low mg/l concentrations to near or at saturation concentrations. In many cases, the range covered two to three orders of magnitude. Thus, the Freundlich adsorption coefficients are appropriate for concentrations found at sites with low as well as with high concentrations of these chemicals.

As discussed, the adsorption potential of a chemical is governed by characteristics of both the chemical and the soil to which it is exposed. The characteristics include: (a) chemical structure, (b) solubility, (c) pH of the solution, (d) ionization potential of the chemical, (e) polarity, and (f) molecular size. The complex interaction of these factors will affect the adsorption that does occur. These


Adsorption of 2,3-Dichlorophenol in Texas Soil at 20° C

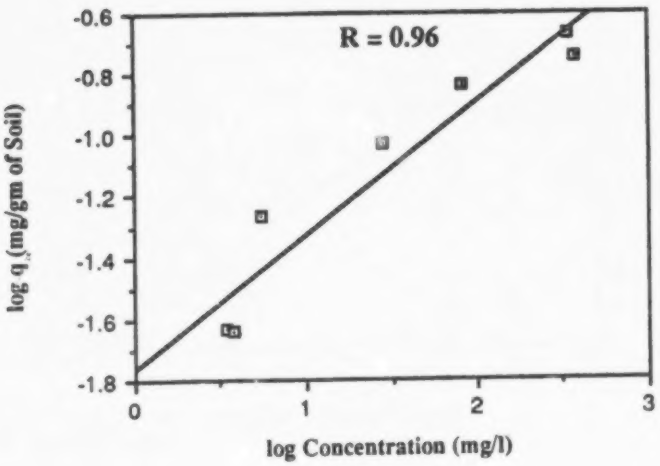
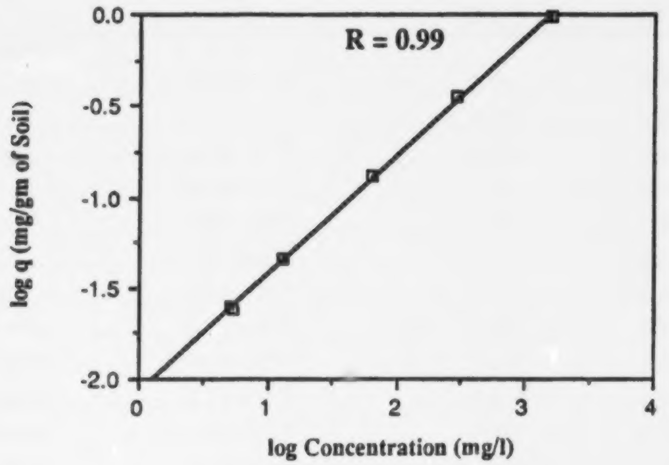

Adsorption of Toluenediamine in Texas Soil at 20° C

Figure 6. Adsorption of 2,3-Dichlorophenol and Toluenediamine in Texas Soil at 20° C

Adsorption of 2,3-Dichlorophenol in Mississippi Soil at 20° C



Adsorption of Toluenediamine in Mississippi Soil at 20° C

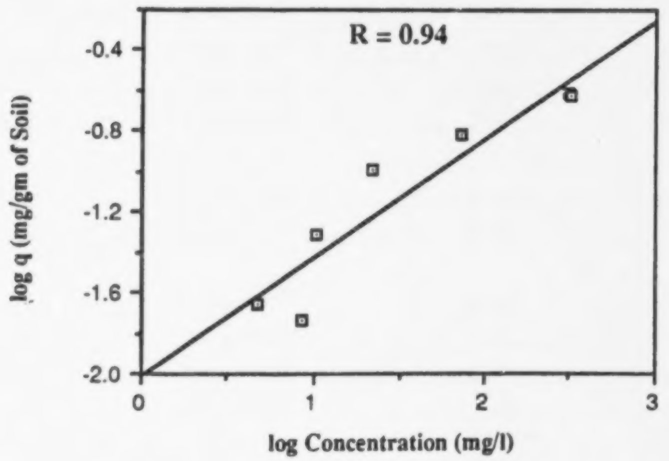


Figure 7. Adsorption of 2,3-Dichlorophenol and Toluenediamine in Mississippi Soil at 20° C.

TABLE 28. BATCH SORPTION ISOTHERM DATA -- TEXAS SOIL FREUNDLICH EQUATION PARAMETERS

COMPOUND	K_F	1/n	r*	PH**
Acid Extractables				
Phenol	3.44×10^{-3}	0.53	0.97	7.9
o-Cresol	2.65×10^{-3}	0.58	0.97	7.9
p-Cresol	9.53×10^{-3}	0.55	0.98	++
m-Cresol	3.19×10^{-6}	0.59	0.97	7.9
2-Chlorophenol	1.01×10^{-3}	0.87	0.99	++
3-Chlorophenol	2.93×10^{-3}	0.76	0.99	7.9
4-Chlorophenol	5.50×10^{-3}	0.72	0.98	7.8
2,3-Dichlorophenol	1.71×10^{-3}	0.91	0.99	7.8
2,4-Dichlorophenol	4.92×10^{-3}	0.77	0.99	7.6
2,5-Dichlorophenol	2.71×10^{-3}	0.82	0.99	8.0
2,6-Dichlorophenol	6.84×10^{-4}	0.90	0.99	7.5
3,4-Dichlorophenol	7.16×10^{-3}	0.75	0.99	8.0
2,4,5-Trichlorophenol	1.46×10^{-3}	1.02	0.96	7.9
2,4,6-Trichlorophenol	1.76×10^{-3}	0.73	0.99	8.1
Pentachlorophenol	5.94×10^{-3}	0.68	0.99	8.1
2,4-Dimethylphenol	3.94×10^{-3}	0.76	0.99	8.1
2-Methyl-4-Chlorophenol	4.97×10^{-3}	0.75	0.99	7.4
3-Methyl-4-Chlorophenol	4.37×10^{-3}	0.79	0.99	7.4
3-Methyl-6-Chlorophenol	1.56×10^{-3}	0.88	0.99	7.4
p-Nitrophenol	3.20×10^{-3}	0.99	0.96	7.4
2,4-Dinitrophenol	1.14×10^{-3}	0.58	0.96	7.7
4,6-Dinitro-o-Cresol	2.30×10^{-3}	0.57	0.96	8.1
Amines				
Toluenediamine	1.74×10^{-2}	0.44	0.96	7.7
Brucine	1.24	0.03	0.47	7.4
Alcohols				
Isobutyl alcohol	1.46×10^{-3}	0.59	0.94	8.0
Allyl alcohol	4.50×10^{-3}	0.45	0.91	8.0
Propargyl alcohol	4.60×10^{-3}	0.73	0.85	8.0
1-Butanol	6.20×10^{-3}	0.42	0.81	8.0
2,3-Dichloropropanol	1.10	0.0004	0.78	++
Methanol	9.30	0.28	0.79	8.0
Other				
2-Nitropropane	3.54×10^{-3}	0.66	0.94	8.0
Thiourea	2.54×10^{-4}	0.81	0.98	8.2

* -- *correlation coefficient;* ** -- *pH of adsorption extract; ++ -- not measured*

TABLE 29. BATCH SORPTION ISOTHERM DATA -- MISSISSIPPI SOIL FREUNDLICH EQUATION PARAMETERS

COMPOUND	K_F	1/n	r*	PH**
Acid Extractables				
Phenol	1.30x10^{-3}	0.71	0.98	5.8
o-Cresol	6.00x$^{10-4}$	0.87	0.98	++
p-Cresol	1.21x10^{-2}	0.47	0.99	++
m-Cresol	8.60x10^{-4}	0.75	1.00	++
2-Chlorophenol	1.60x10^{-3}	0.77	1.00	++
3-Chlorophenol	4.50x10^{-3}	0.63	0.98	5.5
4-Chlorophenol	9.90x10^{-3}	0.55	0.99	5.6
2,3-Dichlorophenol	8.5x10^{-3}	0.65	1.00	6.9
2,4-Dichlorophenol	6.80x10^{-3}	0.65	1.00	++
2,5-Dichlorophenol	6.90x10^{-3}	0.80	0.99	++
2,6-Dichlorophenol	6.70x10^{-3}	0.61	1.00	++
3,4-Dichlorophenol	4.70x10^{-3}	0.78	0.96	5.6
2,4,5-Trichlorophenol	3.60x10^{-3}	0.91	0.97	++
2,4,6-Trichlorophenol	9.80x10^{-3}	0.57	1.00	++
Pentachlorophenol	1.6x10^{-3}	0.51	1.00	6.2
2,4-Dimethylphenol	5.10x10^{-5}	1.28	0.92	5.1
2-Methyl-4-Chlorophenol	6.40x10^{-3}	0.65	1.00	++
3-Methyl-4-Chlorophenol	7.30x10^{-3}	0.68	0.99	7.0
3-Methyl-6-Chlorophenol	3.00x10^{-3}	0.77	0.99	6.2
p-Nitrophenol	3.20x10^{-3}	0.64	1.00	5.7
2,4-Dinitrophenol	1.10x10^{-3}	0.78	0.95	++
4,6-Dinitro-o-Cresol	8.60x10^{-4}	0.86	0.99	4.5
Amines				
Toluenediamine	5.07x10^{-5}	1.28	0.94	5.3
Brucine	6.18x10^{-2}	0.48	0.97	++
Alcohols				
Isobutyl alcohol	5.10x10^{-5}	1.28	0.92	5.7
Allyl alcohol	3.30x10^{-4}	0.72	0.96	5.7
Propargyl alcohol	6.30x10^{-4}	0.61	0.89	5.8
1-Butanol	2.80x10^{-3}	0.43	0.85	5.7
2,3-Dichloropropanol	5.0x10^{-5}	0.90	0.64	++
Methanol	1.40x10^{-3}	0.66	0.82	5.9
Other				
2-Nitropropane	3.54x10^{-2}	0.66	0.94	5.7
Thiourea	1.50x10^{-4}	0.92	0.98	5.1

** -- correlation coefficient; ** -- pH of adsorption extract; ++ -- not measured*

TABLE 30. CHEMICAL CONCENTRATION RANGE EVALUATED DURING THE BATCH ADSORPTION EXPERIMENTS -- TEXAS SOIL

Compound	Concentration Range (mg/l)
Acid Extractables	
Phenol	9-10,300
o-Cresol	20-1,000
p-Cresol	11-6,400
m-Cresol	8-3,200
2-Chlorophenol	26-20,300
3-Chlorophenol	27-7,800
4-Chlorophenol	18-8,600
2,3-Dichlorophenol	40-3,900
2,4-Dichlorophenol	19-2,300
2,5-Dichlorophenol	16-3,600
2,6-Dichlorophenol	26-1,500
3,4-Dichlorophenol	21-520
2,4,5-Trichlorophenol	19-910
2,4,6-Trichlorophenol	20-410
Pentachlorophenol	3-13
2,4-Dimethylphenol	90-7,400
2-Methyl-4-Chlorophenol	18-2,400
3-Methyl-4-Chlorophenol	25-3,800
3-Methyl-6-Chlorophenol	36-1,400
p-Nitrophenol	10-5,200
2,4-Dinitrophenol	10-500
4,6-Dinitro-o-Cresol	18-130
Amines	
Toluenediamine	27-550
Brucine	15-300
Alcohols	
Isobutyl alcohol	40-3,600
Allyl alcohol	15-4,300
Propargyl alcohol	45-4,900
1-Butanol	67-3,800
2,3-Dichloropropanol	78-7,600
Methanol	49-4,200
Other	
2-Nitropropane	58-2,800
Thiourea	17-4,800

TABLE 31. CHEMICAL CONCENTRATION RANGE EVALUATED DURING THE BATCH ADSORPTION EXPERIMENTS -- MISSISSIPPI SOIL

Compound	Concentration Range (mg/l)
Acid Extractables	
Phenol	9-10,300
o-Cresol	20-1,000
p-Cresol	12-6,400
m-Cresol	100-13,000
2-Chlorophenol	34-20,300
3-Chlorophenol	25-8,800
4-Chlorophenol	34-8,600
2,3-Dichlorophenol	30-2,600
2,4-Dichlorophenol	18-2,500
2,5-Dichlorophenol	22-2,800
2,6-Dichlorophenol	26-1,500
3,4-Dichlorophenol	18-520
2,4,5-Trichlorophenol	22-930
2,4,6-Trichlorophenol	16-310
Pentachlorophenol	7-13
2,4-Dimethylphenol	18-6,300
2-Methyl-4-Chlorophenol	15-2,500
3-Methyl-4-Chlorophenol	43-3,900
3-Methyl-6-Chlorophenol	12-1,400
p-Nitrophenol	10-5,300
2,4-Dinitrophenol	4-260
4,6-Dinitro-o-Cresol	23-130
Amines	
Toluenediamine	160-580
Brucine	30-300
Alcohols	
Isobutyl alcohol	40-3,700
Allyl alcohol	15-4,300
Propargyl alcohol	45-4,900
1-Butanol	67-3,800
2,3-Dichloropropanol	78-7,600
Methanol	49-4,000
Other	
2-Nitropropane	59-2,800
Thiourea	21-5,000

experiments were conducted under reasonable real world conditions. Soils of different characteristics (pH, CEC and organic carbon) different chemicals, and different chemical concentrations were used. As a result it was not possible to determine the relative effect of such parameters.

However, the data do allow overall effects to be identified. When the Freundlich K_f values for the two soils, i.e., the capacity or affinity of the soils, were compared (Table 32), in general, the Texas soil had the greater values and therefore the greater sorption capacity for the acid extractables. However, the opposite was true for the amines and alcohols for which the Mississippi soil had the greater K_f values, in some cases greater by a factor of 10 or 100.

CONCLUSIONS

1. The Freundlich equation described the adsorption of the chemicals on the two soils satisfactorily, i.e., with high correlation coefficients, except for a few chemicals.
2. The range of chemical concentrations evaluated ranged from the low mg/l concentrations to near or at saturation concentrations and for most chemicals covered two to three orders of magnitude. Thus, the adsorption data are appropriate for concentrations found at sites with low as well as high concentrations of these chemicals.
3. For these concentration ranges, a linear adsorption relationship, i.e., $n = 1$, did not occur.
4. The Freundlich K_f values for chemicals in the two soils were different. For the acid extractables, the K_f values generally were greater in the Mississippi soil. For the amines and alcohols, the K_f values were greater in the Texas soil.

TABLE 32. COMPARISON OF FREUNDLICH ADSORPTION COEFFICIENTS (K_f) FOR THE TEXAS AND MISSISSIPPI SOILS

Compound	Texas Soil	Mississippi Soil
Acid Extractables		
Phenol	3.4×10^{-3}	1.3×10^{-3}
o-Cresol	2.7×10^{-3}	6.0×10^{-4}
p-Cresol	9.5×10^{-3}	1.2×10^{-2}
m-Cresol	3.2×10^{-6}	8.6×10^{-4}
2-Chlorophenol	1.0×10^{-3}	1.6×10^{-3}
3-Chlorophenol	2.9×10^{-3}	4.5×10^{-3}
4-Chlorophenol	5.5×10^{-3}	9.9×10^{-3}
2,3-Dichlorophenol	1.7×10^{-3}	8.5×10^{-3}
2,4-Dichlorophenol	4.9×10^{-3}	6.8×10^{-3}
2,5-Dichlorophenol	2.7×10^{-3}	6.9×10^{-3}
2,6-Dichlorophenol	6.8×10^{-4}	6.7×10^{-3}
3,4-Dichlorophenol	7.2×10^{-3}	4.7×10^{-3}
2,4,5-Trichlorophenol	1.5×10^{-3}	3.6×10^{-3}
2,4,6-Trichlorophenol	1.8×10^{-3}	9.8×10^{-3}
Pentachlorophenol	5.9×10^{-3}	1.6×10^{-3}
2,4-Dimethylphenol	3.9×10^{-3}	5.1×10^{-5}
2-Methyl-4-Chlorophenol	5.0×10^{-3}	6.4×10^{-3}
3-Methyl-4-Chlorophenol	4.4×10^{-3}	7.3×10^{-3}
3-Methyl-6-Chlorophenol	1.6×10^{-3}	3.0×10^{-3}
p-Nitrophenol	3.2×10^{-3}	3.2×10^{-3}
2,4-Dinitrophenol	1.1×10^{-3}	1.1×10^{-3}
4,6-Dinitrophenol	2.3×10^{-3}	8.6×10^{-3}
Amines		
Toluenediamine	1.7×10^{-2}	5.1×10^{-5}
Brucine	1.24	6.2×10^{-2}
Alcohols		
Isobutyl alcohol	1.5×10^{-3}	5.1×10^{-5}
Allyl alcohol	4.5×10^{-3}	3.3×10^{-4}
Propargyl alcohol	4.6×10^{-3}	6.3×10^{-4}
1-Butanol	6.2×10^{-3}	2.8×10^{-3}
2,3-Dichloropropanol	1.10	5.0×10^{-5}
Methanol	9.3	1.4×10^{-3}
Other		
2-Nitropropane	3.5×10^{-3}	3.5×10^{-2}
Thiourea	2.5×10^{-4}	1.5×10^{-4}

SECTION 7

TOXICITY REDUCTION

A major objective of this study was to provide comprehensive screening data on the treatability of specific organic chemicals in soil. Hazardous constituents that enter the soil are to be detoxified or immobilized.

When a chemical is added to the soil, it is transformed into other products through chemical and biological reactions with or without complete detoxification and immobilization. Measuring the loss of the parent compound, such as was presented in Section 4, does not assure that complete detoxification and immobilization occurs. Intermediate degradation products, which may be more mobile and/or toxic than the parent compound, may be generated as the parent compound degrades.

Additional information on the transformation and/or detoxification of a chemical is necessary to establish that the loss of the parent compound leads to the complete detoxification of the chemical or waste. Such information can be obtained using either chemical or bioassay analyses.

Chemical analysis of detoxification products may yield information about biochemical degradation pathways, but it is time consuming and expensive. Bioassays have been used successfully to demonstrate detoxification of the applied waste in the soil(12, 13, 15) and are less expensive and time consuming. Such bioassays also have been used as a screening tool to evaluate the soil treatment potential of a chemical or waste(12, 34).

APPROACH

In this study, the reduction of toxicity that occurred in selected degradation studies was evaluated by determining the toxicity of the water soluble fraction (WSF) of the chemical/soil mixture at the same sampling intervals used to obtain the degradation data. The chemical compounds that can be extracted with water represent the potentially leachable fraction of the chemical or any intermediate chemical detoxification products. The WSF of the chemical poses the greatest threat to groundwater contamination. Hence, evaluating the loss of the potentially leachable fraction of a chemical is important.

In addition, the concentration of the parent chemical in the WSF also was determined. This concentration was expressed in terms of quantity of chemical that was water extractable per kg of the soil. The procedures for: (a) obtaining the WSF, (b) determining the toxicity of the WSF using the Microtox© method, and (c) determining the chemical concentration were the same as those described in the earlier sections.

To put the toxicity reduction data in perspective, the WSF toxicity reductions, the WSF chemical concentration reductions and the soil chemical concentration reductions were compared.

RESULTS

This toxicity and chemical reduction comparison was done for phenol and eight different chloro- substituted phenols: phenol; 2-, 3- and 4-Chlorophenol; 2,3-, 2,4-, and 2,6-Dichlorophenol; 2,4,6-Trichlorophenol; and Pentachlorophenol. Texas soil was used in each degradation study.

Two loading rates were used. One was the acceptable loading rate identified in Section 4 (Table 11) and the other was twice those loading rates. Based upon previous work(12, 13), loading rates twice the acceptable rates were not expected to completely inhibit degradation. However, the higher rates could slow degradation rates and may result in detoxification by-products if incomplete detoxification occurred.

Chemical Loss in Soil

The kinetic parameters for the loss of chemical in the soil microcosms are presented in Table 33. Data that represented concentrations that were zero or below the detectable limit were not included in the kinetic analyses because it was not clear

TABLE 33. CHEMICAL LOSS IN SOIL -- KINETIC PARAMETERS

Chemical	Initial Conc mg/kg+	First Order Kinetics			Zero Order Kinetics		
		Half Life (days)	r*	95 %CI**	mg/kg-d	r	95% CI
Phenol	1400	19.5	0.96	16.5-23.9	32.7	0.95	25.7-39.6
	700	4.1	0.92	3.2-5.7	59.3	0.96	48.5-70.1
2-Chlorophenol	760	4.1	0.97	3.5-4.9	37.0	0.90	24.2-49.8
	380	1.7	0.98	1.5-1.9	26.1	0.90	20.4-32.0
3-Chlorophenol	235	16.2	0.93	13.1-21.5	6.0	0.92	4.4-7.6
	120	21.8	0.97	19.6-24.6	2.3	0.95	2.0-2.6
4-Chlorophenol	176	1.5	0.92	0.9-3.6	21.5	0.92	8.6-34.3
	88	1.0	0.91	0.7-1.3	14.5	0.91	9.7-19.2
2,3-Dichlorophenol	260	27.2	0.91	21.2-37.9	4.7	0.88	3.0-6.3
	130	8.3	0.97	8.1-8.5	3.6	0.98	3.5-3.7
2,4-Dichlorophenol	180	3.7	0.89	2.7-5.8	8.4	0.98	7.2-9.6
	90	1.5	0.94	1.1-2.4	6.1	0.96	5.4-6.8
2,6-Dichlorophenol	1260	12.8	0.99	12.0-13.7	34.3	0.96	27.9-40.7
	630	2.4	0.91	2.0-3.0	41.8	0.97	36.7-46.9
2,4,6-Trichlorophenol	600	11.0	0.92	8.7-15.0	18.0	0.88	11.9-24.2
	300	5.3	0.94	4.60-6.3	10.4	0.94	8.8-12.1
Pentachlorophenol	60	3.6[a]	1.00	-------	4.4[a]	0.88	--------
	30	6.7	0.92	5.5-8.5	0.99	0.90	0.80-1.2

+ *Initial concentration of chemical in soil at time zero, mg/chemical per kg of dry soil, lower concentration is the acceptable loading rate as discussed in Section 4 and as noted in Table 11.*

* *Correlation coefficient*

** *95% confidence interval for the kinetic parameter*

[a] *Data from initial lag phase (through day 21) were excluded in calculating these values; data points were too few to calculate 95% confidence intervals.*

when the chemical actually disappeared. The data fit both first and zero order kinetics satisfactorily as indicated by the high correlation coefficients.

The data for the lower loading rates were the same as that obtained in the degradation studies (Section 5, Table 18) since they were done at the same time as the previous studies. In most cases, the kinetic parameters at the higher loading rates were larger than those at the lower loading rates.

Example chemical loss patterns for 2,4-Dichlorophenol and Pentachlorophenol (PCP) at the higher loading rates are noted in Figure 8. The initial PCP concentration of 60 mg/kg resulted in a lag phase up to 21 days after the chemical loading. However, the microcosm extracted on day 29 indicated a reduced concentration of PCP and that acclimation had occurred. Because there was a rapid chemical loss after the lag period (Figure 8), kinetic parameters were obtained using data from days 21 and 29. No lag periods occurred for the other chemicals at any of the loading rates.

WSF Chemical Loss

The change of chemical concentration in the WSF also was analyzed and both first and zero order kinetic parameters were determined. The kinetic data are summarized in Table 34. First and zero order kinetics satisfactorily represented the data. Due to rapid loss of 4-Chlorophenol, data on the WSF reduction of this chemical were limited. As with the soil data, PCP concentrations in the WSF exhibited a lag phase at the higher loading and only zero order kinetics could be calculated from the data obtained after the end of the lag phase.

With respect to the higher loading rates, Table 34 indicates that: (a) higher chemical concentrations were in the WSF initially at the higher loading rates, and (b) for the first order kinetics, the WSF chemical concentrations decreased at a slower rate (greater half life) when the chemicals were applied at the higher loading rate. No difference was apparent in zero order kinetics due to the difference in loading rates.

Examples of WSF chemical loss patterns for two chemicals are presented in Figure 9. The losses for both initial concentrations are indicated.

TABLE 34. LOSS OF WATER EXTRACTABLE CHEMICAL -- KINETIC PARAMETERS

Chemical	Initial Conc	First Order Kinetics			Zero Order Kinetics		
	mg/kg$^+$	Half Life (days)	r*	95 %CI**	mg/kg-d	r	95% CI
Phenol	1150	10.9	0.80	7.8-18.1	35.5	0.85	23.5-47.5
	575	3.7	0.60	2.0-36.8	46.8	0.87	27.9-65.7
2-Chlorophenol	610	4.0	0.98	3.7-4.4	21.7	0.86	15.3-28.2
	305	2.2	0.95	1.8-2.9	30.6	0.95	23.6-37.6
3-Chlorophenol	155	14.6	0.98	13.2-16.4	4.4	0.95	3.6-5.1
	75	9.5	0.93	7.9-11.9	2.9	0.91	2.3-3.6
4-Chlorophenol	93	1.6	0.99	---#---	11.9	0.95	---#--
	32	1.6	0.97	---#---	5.3	0.74	---#--
2,3-Dichlorophenol	130	17.0	0.89	13.5-22.9	3.2	0.90	2.4-4.0
	68	7.9	0.97	7.0-9.1	3.6	0.95	3.0-4.1
2,4-Dichlorophenol	98	3.3	0.91	2.6-4.6	4.8	0.94	3.8-5.8
	55	0.93	0.85	0.6-1.9	8.8	0.97	7.0-10.6
2,6-Dichlorophenol	1080	9.5	0.92	7.9-12.0	32.4	0.98	30.0-35.8
	580	8.3	0.99	7.7-8.9	27.9	0.97	24.2-31.6
2,4,6-Trichlorophenol	570	11.5	0.92	9.5-14.7	17.0	0.93	13.6-20.4
	310	6.5	0.98	5.9-7.4	16.4	0.98	14.6-18.1
Pentachlorophenol	25	----initial lag phase----			2.2	0.99	-------
	11	3.9	0.84	2.7-7.3	1.2	0.88	0.76-1.7

+ *Milligram of chemical that was water soluble at time zero per kilogram of dry soil, average of two or three replicates*

* *Correlation coefficient*

** *95% confidence interval for the kinetic parameter*

# *Insufficient data to calculate 95% confidence intervals due to rapid loss of chemicals*

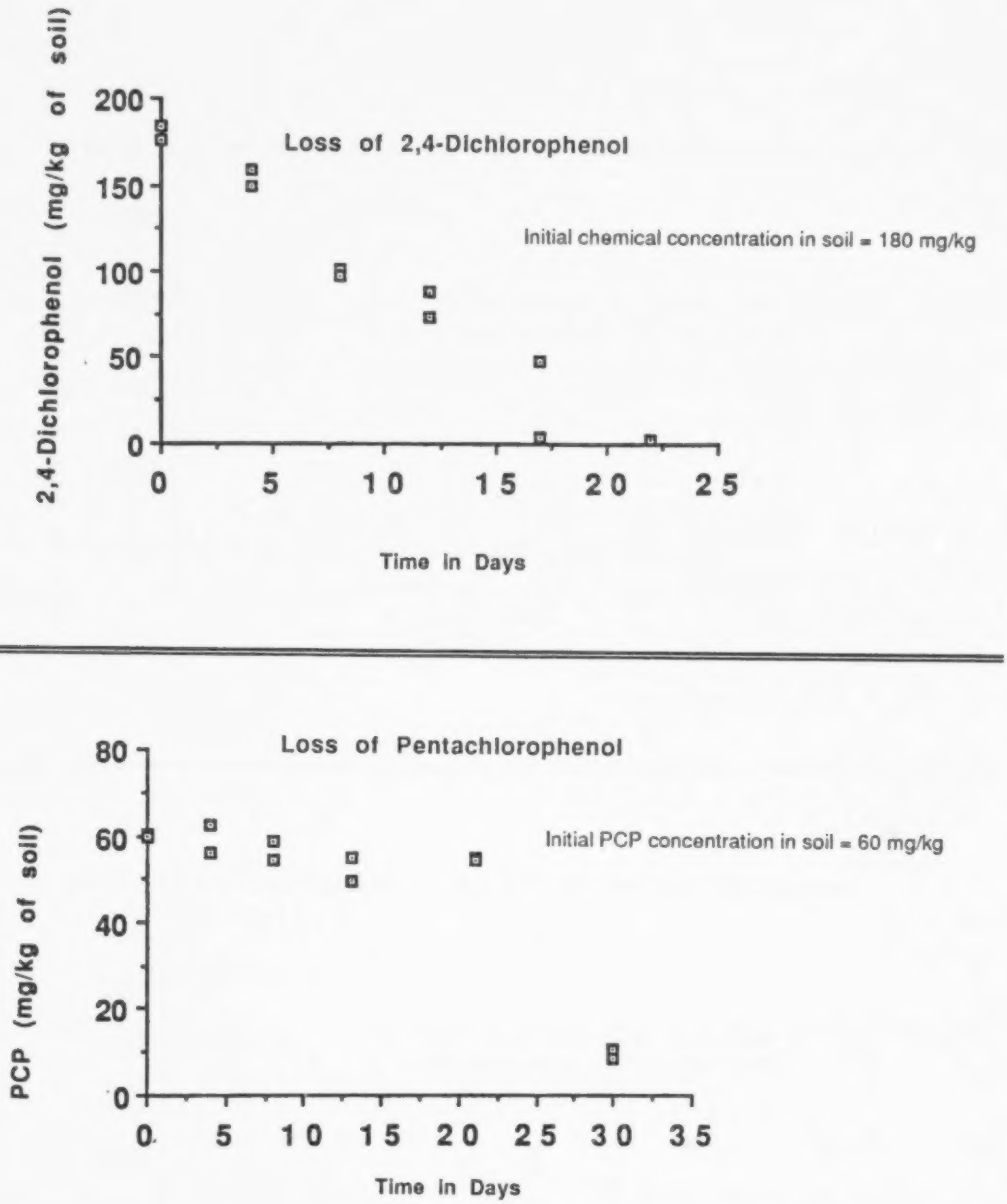
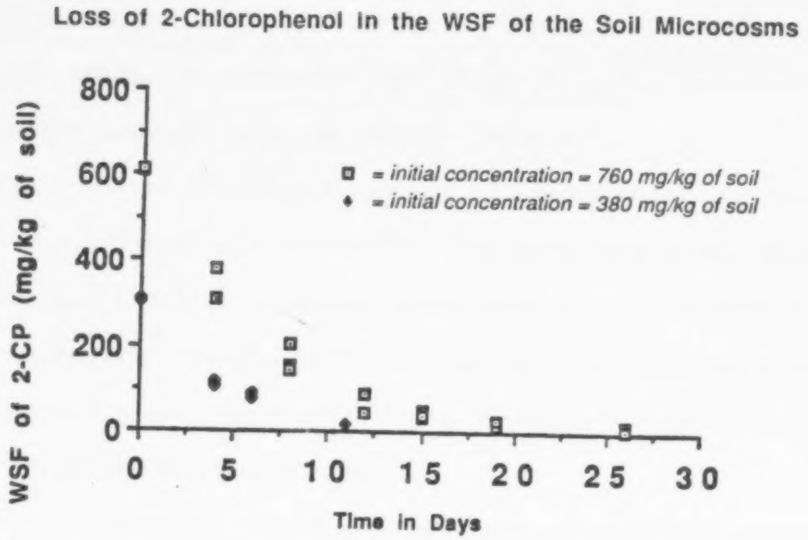
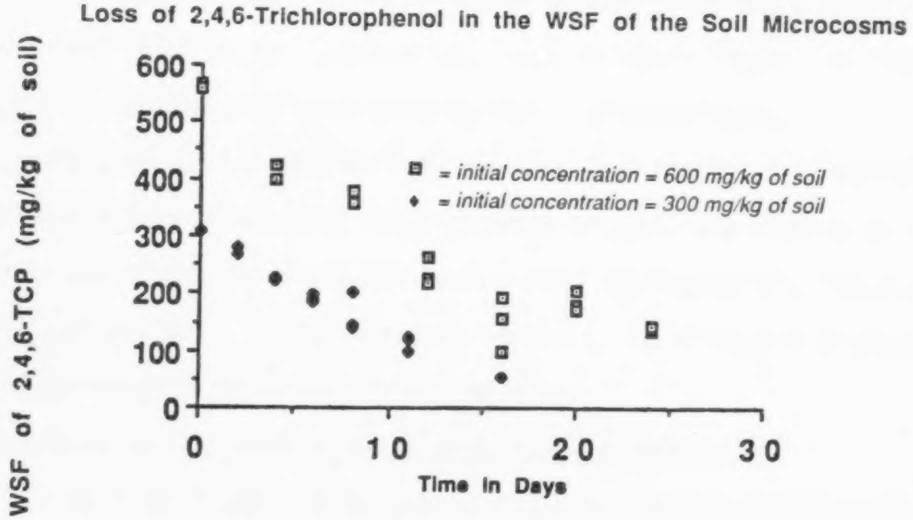


Figure 8. Loss of Chemical in Two Experiments When the Higher Loading Rates Were Used



Loss of 2-Chlorophenol in the WSF of the Soil Microcosms



Loss of 2,4,6-Trichlorophenol in the WSF of the Soil Microcosms

Figure 9. Loss of Chemical in The WSF at Two Loading Rates

WSF Toxicity Reduction

The evaluation of loss of applied chemical in the soil microcosms and in WSF did not address the existence of possible toxic transformation products. A study of the toxicity of the constituents in the WSF can indicate the presence and/or accumulation of any potentially leachable toxic intermediate products.

The toxicity of the WSF was measured by the Microtox$^{©}$ procedure and was expressed as soil toxicity units (Section 4). The resulting data was analyzed by both first and zero order kinetics to provide renoesults that were consistent with the soil chemical loss and the WSF chemical loss. Data on these kinetic parameters, in terms of toxicity reduction, are presented in Table 35. Both first and zero order kinetics appeared to represent the data satisfactorily.

Examples of the toxicity reduction patterns that occurred are presented in Figure 10. The WSF toxicity reductions that were noted were the result of the detoxification that occurred in the soil microcosms as they were incubated at 20° C for the indicated time periods.

With Pentachlorophenol, the WSF toxicity reduction indicated a slow reduction for the first days of incubation, followed by a rapid decrease (Figure 10). No other lag periods in toxicity reduction were observed for the other chemicals.

As had been noticed with the WSF chemical loss and the soil chemical loss, the soil chemical loading rates did cause different results. With respect to the toxicity reduction data (Table 35), the higher loading rates: (a) resulted in a higher initial WSF toxicity, and (b) larger toxicity reduction half-lives. No difference in the zero order kinetics as a function of loading rate was apparent.

Comparison of Chemical Losses and Toxicity Reduction

The loss of the chemical in the soil and in the WSF and the WSF toxicity reduction data were compared using the first order kinetic data (half-lives). Figure 11 compares the half-life of the soil and the WSF chemical loss for all of the nine chemicals tested at both low and high loadings. The correlation shows that, in general, the soil chemical half-life was about 1.5 times greater than the WSF chemical half-life. For these chemicals, this indicates that the loss of the chemical in the WSF was about 1.5 times faster than the loss of the chemical in the soil. This correlation also indicated that no enhanced mobilization of applied chemical occurred as the degradation and detoxification took place.

TABLE 35. WSF TOXICITY REDUCTION -- KINETIC PARAMETERS

			First Order Kinetics			Zero Order Kinetics		
Chemical	Chemical Initial Conc mg/kg$^+$	Initial Toxicity TU^{++}	Half Life (days)	r*	95 %CI**	Soil TU/d	r	95% CI
Phenol	1400	40	14.9	0.87	11.6-20.6	1.17	0.90	0.89-1.5
	700	13	10.2	0.5	----	1.17	0.81	0.65-1.7
2-Chlorophenol	760	20	5.8	0.96	5.0-7.0	0.90	0.91	0.69-1.7
	380	10	5.8	0.95	4.6-7.8	0.76	0.95	0.57-0.95
3-Chlorophenol	235	28	14.5	0.88	11.5-19.5	0.76	0.92	0.60-0.91
	120	15	8.9	0.91	7.3-11.3	0.62	0.95	0.52-0.95
4-Chlorophenol	176	46	1.8	0.87	1.1-4.0	6.0	0.90	3.0-9.0
	88	6	0.6	0.97	--@--	4.5	0.88	2.3-6.6
2,3-Dichlorophenol	260	37	18.7	0.92	15.5-23.6	0.75	0.89	0.56-0.94
	130	19	7.7	0.90	6.2-10.2	1.1	0.89	0.8-1.3
2,4-Dichlorophenol	180	52	5.3	0.95	4.5-6.5	2.2	0.98	2.0-2.5
	90	22	2.4	0.87	1.7-4.1	2.9	0.93	2.1-3.8
2,6-Dichlorophenol	1260	31	11.2	0.95	9.6-13.4	0.95	0.95	0.80-1.1
	630	14	13.3	0.79	9.3-23.7	0.49	0.81	0.29-0.70
2,4,6-Trichlorophenol	600	29	12.1	0.92	9.9-15.3	0.79	0.89	0.59-0.99
	300	12	5.8	0.94	5.0-7.1	0.64	0.94	0.53-0.76
Pentachlorophenol	60	31	24.3	0.78	16.4-46.6	0.65	0.76	0.31-1.0
	30	13	7.3	0.88	5.3-11.9	0.90	0.88	0.55-1.3

+ *Initial concentration of chemical in soil at time zero, mg/chemical per kg of dry soil, lower concentration is the acceptable loading rate as discussed in Section 4 and as noted in Table 11*

++ *Toxicity of the WSF at time zero in toxicity units (Section 4)*

* *Correlation coefficient*

** *95% confidence interval for the kinetic parameter*

a *Data from initial lag phase (through day 21) were excluded in calculating these values; data points were too few to calculate 95% confidence intervals.*

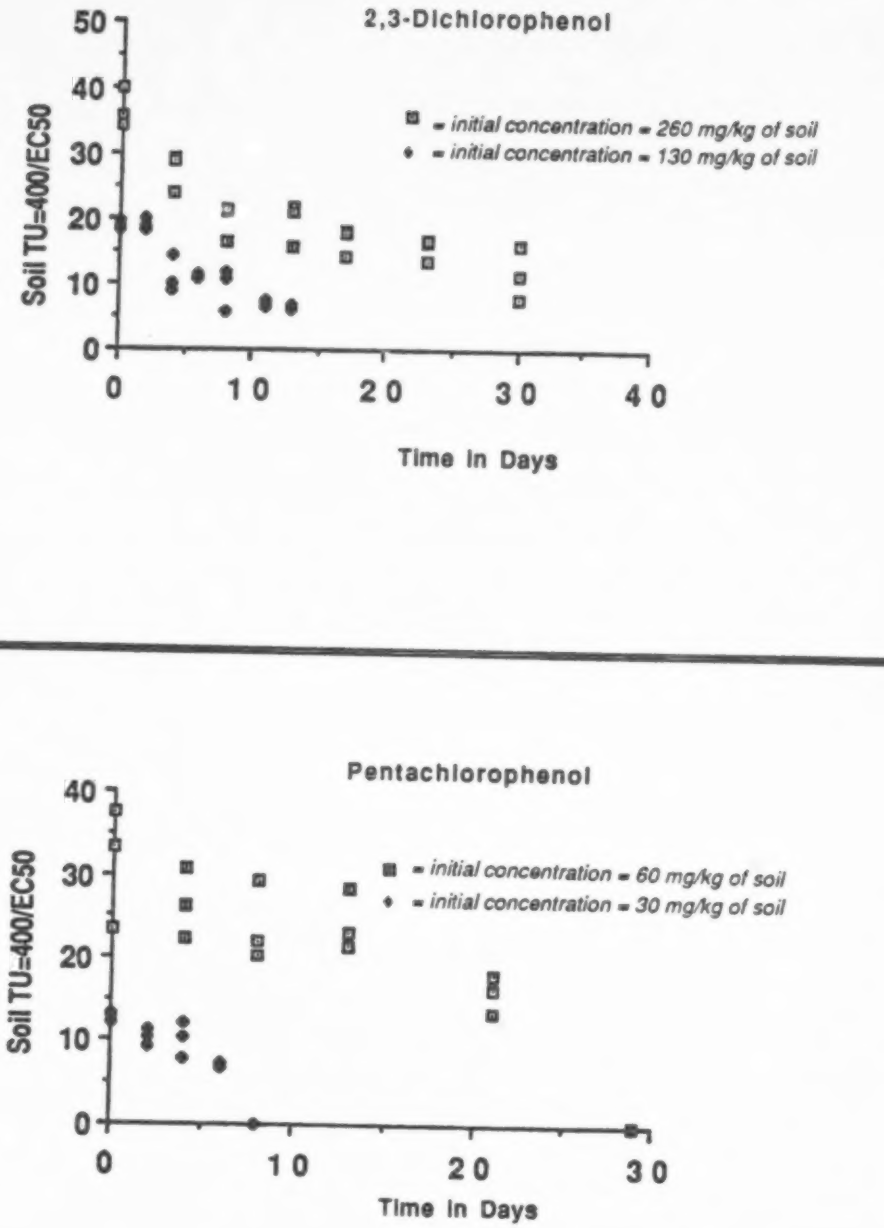


Figure 10. Toxicity Reduction in the WSF from the Soil Microcosms

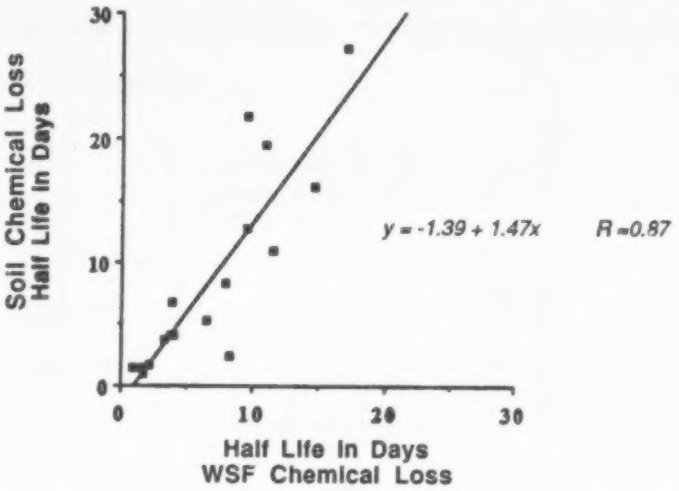


Figure 11. Comparison of Chemical Loss in the Soil and the WSF for Phenol and Eight Chlorophenols

The toxicity reduction study was conducted to evaluate the possibility of toxic intermediate chemicals and their effect, if any, on the treatment of the chemical applied to the soil. The WSF of the land applied hazardous constituents pose the immediate threat to soil microbes and groundwater, and the identification of any potentially leachable toxic constituents is important to evaluate the performance of a HWLT.

The toxicity of the WSF could be a result of: (a) the chemical added to the soil, (b) intermediate transformation products that are potentially leachable, and (c) background toxicity from the soil. The analyses of blank samples (soil only) did not show any background toxicity from the Texas soil used in this research. The toxicity of the WSF extracted on day zero was contributed only by the applied target chemical, since degradation had not yet occurred. The subsequent reduction in toxicity of the WSF was a result of detoxification and immobilization reactions in the soil.

The chemical loss in the soil and the WSF and the WSF toxicity reduction were compared. In each case, the WSF toxicity decreased as the soil chemical and the

WSF chemical concentrations decreased. Figure 12 provides an example of the decreases for 2,4-Dichlorophenol.

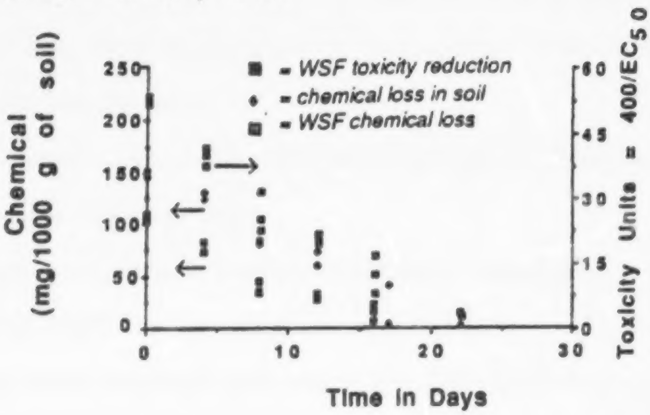


Figure 12. Decrease of Soil and WSF Chemical Concentration and of WSF Toxicity as a Function of Time-Degradation Study of 2,4-Dichlorophenol

Figure 13 compares the WSF chemical loss and the WSF toxicity reduction for all nine tested chemicals at both low and high loadings. The correlation of 0.90 indicates that the WSF toxicity can be attributed to the target chemical concentration in the WSF and that no water extractable toxic intermediate products were formed. Thus, these chemicals were detoxified in the soil.

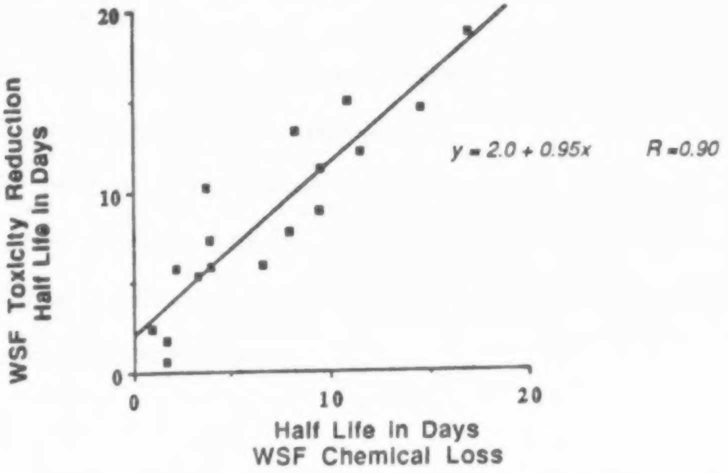


Figure 13. Comparison of WSF Chemical Loss and the WSF Toxicity Reduction for Phenol and Eight Chlorophenols

CONCLUSIONS

The loss of chemical in the soil and in the WSF extracted from the soil and the reduction of toxicity of the WSF were evaluated for nine chemicals that were part of this study: phenol and eight chlorophenols. Two loading rates and one soil (Texas soil) were used. The results were:

1. Both first and zero order kinetics satisfactorily represented the chemical loss and toxicity reduction data.
2. The microcosms with Pentachlorophenol resulted in a lag phase in chemical loss and WSF toxicity reduction, especially at the higher loading rate. No lag periods were observed with any of the other chemicals at either loading rate.
3. The higher chemical loading rates resulted in higher chemical concentrations in the WSF and higher WSF toxicities at time zero.
4. The higher chemical loading rates generally resulted in slower chemical losses (higher half-lives) and toxicity reduction. However, at both loading rates for each chemical, the chemicals were degraded and the toxicity was reduced.
5. No differences were apparent in zero order kinetics due to the loading rates.
6. The loss of the chemicals in the WSF was about 1.5 times faster than the loss of the chemical in the soil.
7. The WSF toxicity for each chemical decreased as the soil chemical and the WSF chemical concentrations decreased.
8. The WSF toxicity decreased at about the same rate as the WSF chemical concentration when the data for all the nine chemicals were compared.
9. No enhanced mobilization of the applied chemical occurred as the degradation and detoxification occurred.

SECTION 8

MUNITIONS WASTES AND CHEMICALS

As part of this cooperative agreement, the soil treatablility potential of a hazardous waste generated in the explosives industry and several chemicals used in that industry were evaluated (Table 2). The toxicity and adsorption behavior of these chemicals and the waste were determined using procedures outlined in Sections 3 and 4. Because of limited quantities of RDX and HMX available, these compounds were not evaluated as part of the degradation studies. RDX and HMX were obtained from the United States Army Toxic and Hazardous Materials Agency (USATHAMA). TNT was obtained commercially from Chem Services, Inc. in Pennsylvania. 2,4- and 2,6-Dinitrotoluene were purchased from Aldrich Chemicals, Milwaukee, Wisconsin.

In addition to these chemicals, wastewater treatment sludge from the manufacturing of explosives at the Holston Army Ammunition Plant was evaluated for its land treatability potential. This sludge was obtained with the help of USATHAMA. The following sections summarize the toxicity, adsorption and degradation characteristics of the pure compounds and the land treatability potential of munitions waste treatment sludge.

RELATIVE TOXICITY AND LOADING EVALUATION

Results of the toxicity evaluation are summarized in Table 36. As part of the adsorption experiments, the maximum solubility of HMX was established as 3.8 mg/l. This low solubility made the toxicity evaluation difficult. The toxicity of hydrophobic compounds cannot be properly evaluated using the Microtox© system. Because of the limited quantities of HMX and RDX that were obtained, neither chemical was able to be evaluated for soil mass loading ranges.

TABLE 36. EC_{50} DATA AND ACCEPTABLE LOADING RATES FOR MUNITIONS MANUFACTURING CHEMICALS

	EC_{50} Value (mg/l)		Loading Rates (mg/kg of soil)+	
Compound	Value	95% CI	Texas Soil	Mississippi Soil
2,4-Dinitrotoluene	31.2	29.7-32.8	500	165
2,6-Dinitrotoluene	4.4	4.3-4.5	86	74
TNT (2,4,6-Trinitrotoluene)	1.0	0.7-1.3	14	12
RDX	76.1	60.0-97.0	*	*
HMX	**	**	*	*

* *Loading rate data for RDX and HMX could not be evaluated since sufficient quantities of material could not be obtained.*

** *Due to its insolubility, EC_{50} data for HMX could not be obtained.*

+ *Lower loading rate as determined using the procedure in Table 9*

ADSORPTION

Methods

The procedure followed for evaluation of the adsorption behavior of RDX, HMX, and TNT was a modification of that outlined in Section 3. The following section describes the protocol used to evaluate the sorption behavior of these chemicals.

RDX and HMX were received as aqueous solutions consisting of approximately 100 mg of chemical in five milliliters of water. Prior to use, the compounds were dried at 105° C. After drying, each chemical was used to prepare standard stock solutions using a 50:50 mixture of water:Methanol as the solvent. This protocol improved the accuracy of chemical detection. The final concentrations of the RDX and HMX stock solutions were 41.8 and 3.8 mg/l, respectively. The resulting concentration of HMX was very close to the detection limit of this compound by high pressure liquid chromatography (~1 mg/l).

TNT was received as a solid consisting of 20% moisture. The moisture content was taken into account in preparing a stock solution of 700 mg/l TNT using a 50:50 mixture of water:methanol as the solvent.

External standard solutions were prepared from the stock solutions by a serial dilution technique utilizing the 50:50 water:methanol solvent mixture. These external standards were used to evaluate the concentration of aqueous chemical stock solutions used in adsorption tests. Chemical concentrations were determined using the HPLC and a 50:50 water:methanol solution as the eluent for the RDX and HMX. Methanol was used as the eluent for TNT.

Results

The range of chemical concentrations evaluated is noted in Table 37. The adsorption results for 2,4- and 2,6-Dinitrotoluene are presented in Table 38. The Freundlich equation described the sorption of these chemicals on the two soils satisfactorily, i.e., with high correlation coefficients. However, such was not the case for the other munitions chemicals.

The sorption data for TNT, RDX and HMX are presented in Tables 39 and 40. With TNT, for both soils, the value of q_e reached a maximum of about 0.045 mg TNT/gm dry soil at a solution concentration of about 15 mg/l TNT and then decreased at higher solution concentrations. This is not in agreement with the Freundlich equation in which q_e should increase as the compound concentration approaches its solubility limit(31). Data for the other chemicals (RDX and HMX) also produced poor correlation with the Freundlich equation. The correlation coefficients ranged from 0.0 to 0.44.

As discussed in Section 6, the adsorption of a chemical is affected by a number of characteristics, none of which were evaluated in these experiments. For the two chemicals for which the Freundlich equation did seem to fit the adsorption data (Table 38), the Texas soil appeared to have a greater affinity (higher K_f value) for 2,4-

TABLE 37. CHEMICAL CONCENTRATION RANGE EVALUATED DURING THE BATCH ADSORPTION EXPERIMENTS -- MUNITIONS CHEMICALS

	Concentration Range (mg/kg)	
Compound	**Texas Soil**	**Mississippi Soil**
2,4-Dinitrotoluene	32-160	15-150
2,6-Dinitrotoluene	37-600	14-200
(2,4,6-Trinitrotoluene) TNT	0.1-60	0.3-90
RDX	8-30	11-32
HMX	0.9-3.6	1.5-3.8

TABLE 38. FREUNDLICH ISOTHERM DATA -- TEXAS AND MISSISSIPPI SOILS -- 2,4- AND 2,6-DINITROTOLUENE

Compound	k_f	**1/n**	**r***	**pH**
Texas Soil				
2,4-Dinitrotoluene	9.2×10^{-3}	0.59	0.88	7.6
2,6-Dinitrotoluene	5.0×10^{-3}	0.61	0.90	8.1
Mississippi Soil				
2,4-Dinitrotoluene	6.3×10^{-3}	1.08	0.99	5.2
2,6-Dinitrotoluene	1.4×10^{-2}	0.68	0.95	5.6

**correlation coefficient*

TABLE 39. ADSORPTION DATA FOR TNT, HMX AND RDX IN TEXAS SOIL AT 20° C

2,4,6-Trinitrotoluene (TNT) (pH=7.8)

Concentration (mg/l)	q_e (mg sorbed/ g dry soil)
0.1	0.0031
6.5	0.0238
13.2	0.0475
60.8	0.0311

RDX (pH=8.0)

Concentration (mg/l)	q_e (mg sorbed/ g dry soil)
8.6	0.0077
10.7	0.0056
11.7	0.0151
13.7	0.0106
26.6	0.0154

HMX (pH=7.9)

Concentration (mg/l)	q_e (mg sorbed/ g dry soil)
0.9	0.00322
1.6	0.00086
2.3	0.00322
3.6	0.00036

TABLE 40. ADSORPTION DATA FOR TNT, HMX AND RDX IN MISSISSIPPI SOIL AT 20° C

2,4,6 Trinitrotoluene (pH=5.8)

Concentration (mg/l)	q_e (mg sorbed/ g dry soil)
0.4	0.00275
10.6	0.0197
17.9	0.0428
90.7	0.0012

RDX (pH=5.8)

Concentration (mg/l)	q_e (mg sorbed/ g dry soil)
11.3	0.00498
11.9	0.00439
12.7	0.0142
19.2	0.00506
32.3	0.00973

HMX (pH=5.9)

Concentration (mg/l)	q_e (mg sorbed/ g dry soil)
1.5	0.00092
1.7	0.00069
3.8	0.00022

Dinitrotoluene while the Mississippi soil appeared to have a greater affinity for the 2,6-Dinitrotoluene.

DEGRADATION STUDIES

The experimental procedures for these degradation studies were identical to those described in Section 5. The degradation of RDX and HMX could not be evaluated due to the limited quantities that were available.

The recovery efficiencies and the loadings that were used in these degradation studies are presented in Table 41. Even though the loading rate for 2,4-Dinitrotoluene was in the acceptable range, no loss of this chemical occurred over a 47-day study. However, losses of 2,6-Dinitrotoluene and 2,4,6-Trinitrotoluene (TNT) did occur. The loss pattern for 2,6-Dinitrotoluene in both soils is presented in Figure 14. The loss rates for the two chemicals are indicated in Table 42.

The data indicate that first order kinetics were a better representation for TNT than were zero order kinetics. The data also indicate that the first order half-life of TNT in the Mississippi soil was less, and the loss faster, than in the Texas soil. No differences in the loss rates for 2,6-Dinitrotoluene for the two soils were apparent.

MUNITIONS WASTEWATER TREATMENT SLUDGE

In addition to the compounds noted above, the land treatability potential of wastewater sludge resulting from the manufacture and processing explosives at the Holston Army Ammunition Plant (HAAP) was evaluated. Figure 15 indicates the wastewater treatment process flow diagram at HAAP. The sludge evaluated was obtained from the Area B raw wastewater settling basin. Prior to use, the characteristics of this sludge were determined.

Nitrogen and COD -- Nitrogen and COD concentrations of the sludge (Table 43) were determined according to procedures described in Standard Methods(36).

TABLE 41. LOADING RATE AND RECOVERY EFFICIENCY DATA FROM THE MUNITIONS CHEMICAL DEGRADATION STUDIES

Compound	Loading Rate (mg/kg of soil) Acceptable+	Loading Rate (mg/kg of soil) Actual	Average Recovery Efficiency (%)++
Texas Soil			
2,4-Dinitrotoluene	500	500	100
2,6-Dinitrotoluene	86	85	91
2,4,6-Trinitrotoluene	14	14	100
Mississippi Soil			
2,4-Dinitrotoluene	165	150	92
2,6-Dinitrotoluene	74	70	85
2,4,6-Trinitrotoluene	12	12	101

+ *data from Table 36*

++ *average of three replicates*

TABLE 42. CHEMICAL LOSS RATE DATA FOR 2,6-DINITROTOLUENE AND TNT IN THE DEGRADATION STUDIES

COMPOUND	KINETIC PARAMETERS FIRST ORDER HALF LIFE (day)	r*	95% C.I.	ZERO ORDER mg/Kg/day	r*	95% C.I
Texas Soil						
2,6-Dinitrotoluene	72	0.91	59-90	0.7	0.91	0.6-0.9
2,4,6-Trinitrotoluene	7.7	0.94	6.5-9.4	0.5	0.81	0.3-0.6
Mississippi Soil						
2,6-Dinitrotoluene	92	0.78	66-153	0.5	0.80	0.3-0.6
2,4,6-Trinitrotoluene	5.7	0.93	4.6-7.2	0.4	0.80	0.2-0.6

* *correlation coefficient*

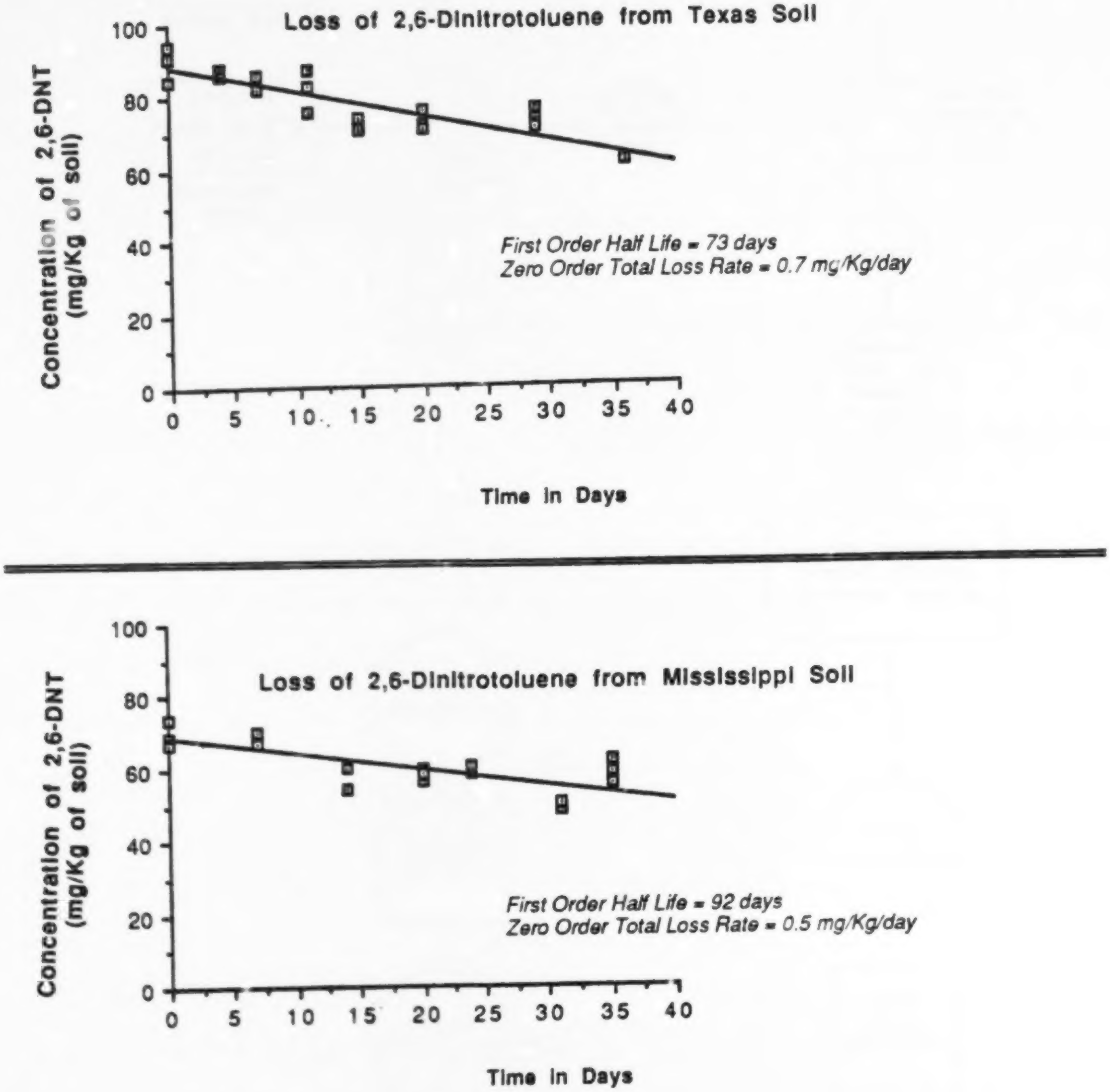


Figure 14. Loss of 2,6-Dinitrotoluene in Texas and Mississippi Soils at 20° C

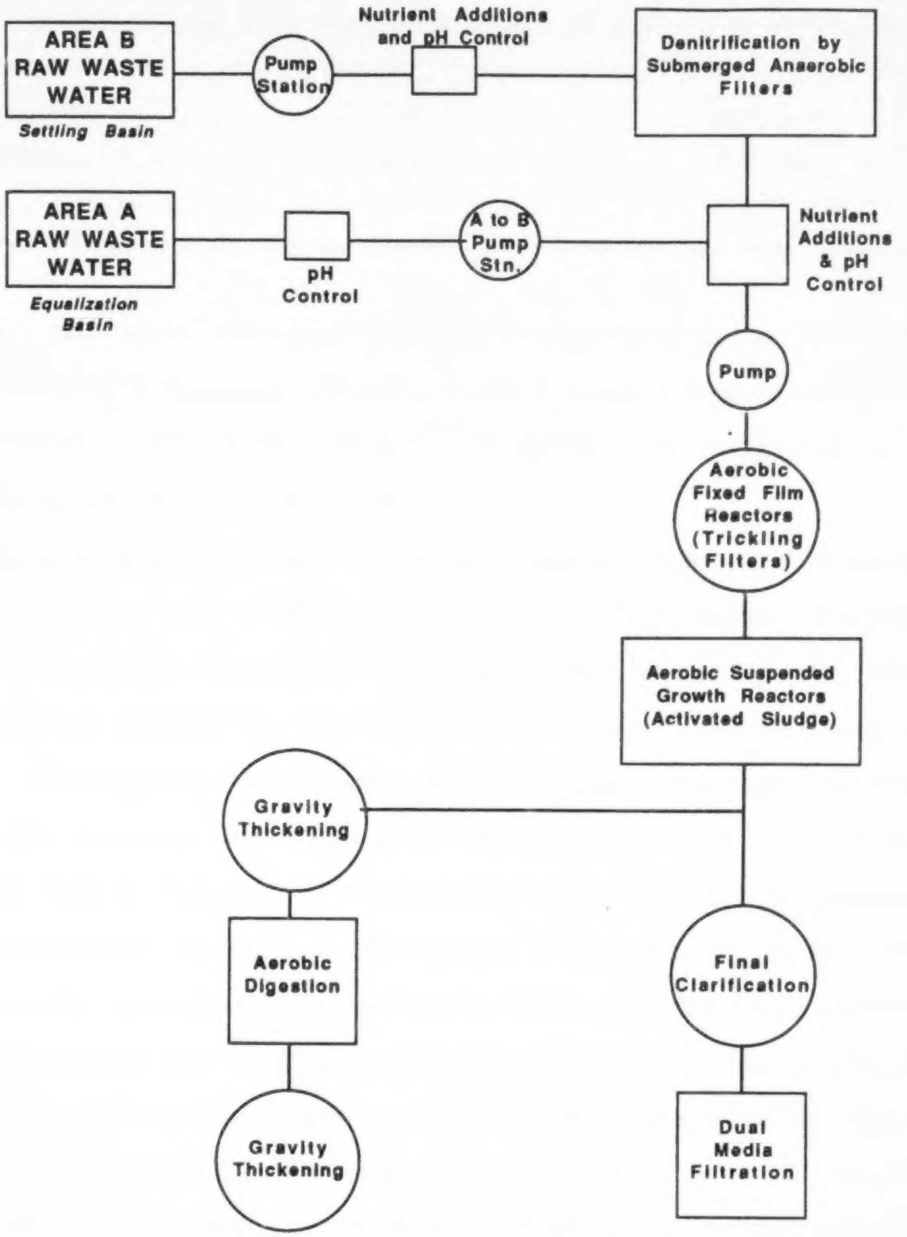

AREA B
RAW WASTE
WATER
Settling Basin
Pump
Station
Nutrient Additions
and pH Control
Denitrification by
Submerged Anaerobic
Filters
AREA A
RAW WASTE
WATER
Equalization
Basin
pH
Control
A to B
Pump
Stn.
Nutrient
Additions
& pH
Control
Pump
Aerobic
Fixed Film
Reactors
(Trickling
Filters)
Aerobic Suspended
Growth Reactors
(Activated Sludge)
Gravity
Thickening
Aerobic
Digestion
Gravity
Thickening
Final
Clarification
Dual
Media
Filtration

Figure 15. Wastewater Treatment Process Flow Diagram for the Holston Army Ammunition Plant(35)

TABLE 43. NITROGEN AND COD CONCENTRATIONS OF MUNITIONS WASTE SLUDGE

Total Kjeldahl Nitrogen	4820 mg/L
Ammonia Nitrogen	2300 mg/L
C.O.D.	1.27×10^5 mg/L

Metals -- The metals in the munitions sludge were analyzed at the USEPA Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma. Results were obtained using procedures outlined in EPA SW 846(7) for both the total sludge and the liquid fraction. The results are contained in Table 44.

GC/MS Analysis -- One goal of this study was to determine if quantities of hazardous organics were present in the munitions waste sludge. If significant quantities were present, degradation experiments would be conducted to estimate loss rates of these constituents. The organics present in the sludge were determined as follows. The organics were extracted from the sludge using a shake extraction procedure and Methylene Chloride as the extracting solvent (EPA method 3450)(7). The extract was analyzed on a Finnigan-MAT4000 gas chromatograph/mass spectrometer (GC/MS) located in the Department of Chemistry at The University of Texas at Austin. Results are summarized in Table 45. The listed compounds represent the fourteen most significant peaks of the chromatogram. Many small peaks were observed but they had a peak intensity of the same magnitude as instrument noise. None of the small peaks corresponded to TNT, HMX or RDX. Because of the absence of these compounds in the munitions waste sludge, degradation experiments with the sludge were not conducted.

Relative Toxicity -- The toxicity of the munitions sludge was evaluated using the Microtox© procedure. It is possible that the chemicals contained as part of the sludge solids could cause toxicity as the solids decomposed. To determine if the

TABLE 44. METALS IN MUNITIONS SLUDGE AND SLUDGE FILTRATE

	Filtrate		Total Sample	
Element	mg/l	Std. Dev.	mg/kg wet wt.	Std. Dev.
Na	20.7	2.0	92.8	9.2
K	18.8	1.9	202	20
Ca	45.8	4.5	711	71
Mg	12.4	1.2	196	1.9
Fe	400	40	3940	390
Mn	1.10	0.09	14.7	1.3
Co	0.02	0.01	0.81	0.15
Mo	<.01	–	0.69	0.26
Al	<.1	–	1740	170
As	<.03	–	5.8	2.50
Se	<.1	–	<1	–
Cd	<.003	–	0.19	0.04
Be	<.003	–	0.21	0.04
Cu	0.03	0.01	13.9	1.3
Cr	0.01	0.01	7.85	0.77
Ni	0.01	0.01	2.85	0.28
Zn	0.10	0.01	35.9	3.6
Ag	<.03	–	<.3	–
Tl	<.04	–	<.7	–
Pb	<.02	–	10.4	1.5
Li	<.01	–	1.15	0.14
Sn	0.17	0.01	3.59	0.35
V	<.03	–	4.76	0.71
Ba	0.01	0.01	11.0	1.1
B	0.23	0.03	0.88	0.31
Ti	<.1	–	6.7	1.4

TABLE 45. GC/MS ANALYSIS OF MUNITIONS SLUDGE

COMPOUND	MOLECULAR WT.	% FIT*	PEAK INTENSITY**
6-Methyl, 1-H Indole C_9H_9N	131	93.8	268
Benzenemethanimine C_7H_7N	105	89.1	81
1,3 Benzoxazine $C_{16}H_{17}ON$	239	73.1	402
8-Methyl, Decanoic Acid $C_{12}H_{24}O_2$	200	79.0	338
2,2-Dimethylcyclohexanol $C_8H_{16}OCl$	128	83.8	172
10-Undecenoyl Chloride $C_{11}H_{19}OCl$	202	84.3	170
8-Methyl Decanoic Acid $C_{12}H_{24}O_2$	202	87.3	290
1-Methyl, 4-Nitrosopiperazine $C_5H_{11}ON_3$	129	70.2	242
Sulfur S_8	256	84.8	641
Undecenal $C_{11}H_{20}O$	168	82.7	148
7-Methyl, Nonanoic acid $C_{11}H_{22}O_2$	186	85.4	240
Hexanedioic Acid $C_{14}H_{26}O_4$	258	84.1	223
3-Nitro, 1,2-Benzene-Dicarboxylic Acid $C_8H_5O_6N$	211	85.7	443
Thiocyanic Acid $C_{11}H_{13}ONS$	207	81.[illegible]	254

* *The % fit refers to how well the library fit matched the ion chromatograph generated from the sample.*

** *Peak intensity refers to the relative concentrations of the compounds in the extract. For example, sulfur (S_8) had the largest concentration of the fourteen compounds listed.*

solids may have included chemicals that exhibited a toxic effect, one set of samples was homogenized and the other was not before the toxicity was determined. With the Microtox© procedure, the soluble constituents exert the greatest effect.

Preparation of both sets of samples consisted of taking aliquots of homogenized and aliquots of nonhomogenized sludge (100 ml each), diluting them with distilled deionized water to obtain different concentrations of the sludge constituents and centrifuging them at 2000 rpm for 15 minutes. Fifty milliliters of the supernatant liquid from each sample were discarded and replaced by fifty milliliters of a 2% Sodium Chloride/distilled deionized water solution. The addition of the salt solution maintained the proper environment for the marine bioluminescent bacteria used in the toxicity test. This procedure was in accordance with standard Microtox© operating methods (Section 4). The samples were then centrifuged at 2000 rpm for an additional 15 minutes. Twenty five milliliters of the supernatant were withdrawn from each sample and filtered (0.45 µm pore size filter). Within thirty minutes after filtering, the samples went from clear to a brownish red color suggesting the oxidation of some metal species. Because of the color interference, a color absorbance correction was necessary to obtain toxicity results. The results of the toxicity evaluation are summarized in Table 46.

As noted above, the homogenized and nonhomogenized sludge samples were diluted to provide varying concentrations of sludge constituents for the toxicity evaluation. This resulted in the toxicity results being known in terms of percent of the original sludge. If the sludge were nontoxic, the EC_{50} value would be around 100%. These evaluations (Table 46) indicated that high dilutions were needed to obtain EC_{50} values and that, therefore, the sludge was toxic to the Microtox© organisms.

Because these samples demonstrated significant toxicity, a second set of samples was processed in the same way and the relative toxicity determined to verify the previous results. The results from this second evaluation were comparable to those presented in Table 46.

TABLE 46. MUNITIONS WASTE TOXICITY DATA -- UNDILUTED SAMPLES

Sample	H&C(1)*	H&C(2)*	C(1)	C(2)
EC_{50} (5 min., 15° C)	0.92%	1.30%	1.22%	1.47%
95% confidence interval	0.52 -1.65%	1.25 -1.35%	1.16 -1.28%	1.37 -1.57%
pH of the sample	5.6	5.7	5.7	5.7

* *H -- homogenized, C -- centrifuged; (1) and (2) are replicates.*

The relative toxicity results indicated that: (a) the munitions sludge exhibited considerable relative toxicity, and (b) there was no difference in relative toxicity between the homogenized and the nonhomogenized samples. The latter statement indicates that the constituents causing the toxicity effect were in the soluble and not the solid phase. The low pH of the sludge (Table 46) may have contributed to the relative toxicity.

CONCLUSIONS

1. The Freundlich equation described the sorption of 2,4- and 2,6-Dinitrotoluene in the two soils satisfactorily. However, it did not do so for TNT, RDX, or HMX.
2. No loss of 2,4-Dinitrotoluene occurred over a 47-day study, even though the loading rate used was determined to be acceptable using procedures discussed in Section 4.
3. Because of the small amounts of RDX and HMX that were received, no degradation studies could be conducted.

4. Degradation loss rates could be obtained for 2,6-Dinitrotoluene and TNT. First order kinetics were a better representation for TNT than were zero order kinetics.
5. The half-life of TNT in the Mississippi soil was shorter, and the loss faster, than in the Texas soil. No differences in the loss rates in the two soils for 2,6-Dinitrotoluene were apparent.
6. The sludge resulting from the manufacture and processing of explosives contained: (a) high concentrations of nitrogen and COD, (b) concentrations generally less than 10 mg/l for the heavy metals, and (c) no amounts of TNT, RDX, or HMX.
7. The munitions sludge had a high toxicity as measured by the Microtox© procedure. The constituents causing the relative toxicity were in the soluble and not the solid phase of the sludge.

SECTION 9

REFERENCES

1. Short, Thomas E. "Modeling of Processes in the Unsaturated Zone". In: R.C. Loehr and J.F. Malina, Jr., Editors, **Land Treatment: A Hazardous Waste Management Alternative**. Water Resources Symposium 13, Center for Research in Water Resources, The University of Texas at Austin, Austin, Texas 78712, 1986, pp. 211-240.

2. Nofziger, D.L., Williams, J.R. and T.E. Short. "Interactive Simulation of the Fate of Hazardous Chemicals During Land Treatment of Oily Wastes: RITZ Users' Guide". Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, Oklahoma, 1988, 59 pages.

3. Caupp, C.L., Grenney, W.J., and P.J. Ludvigsen. "VIP -- A Model for the Evaluation of Hazardous Substances in Soil -- Version 2". Civil and Environmental Engineering Department, Utah State University, Logan, Utah, 1988, 25 pages.

4. Grenney, W.J., Caupp, C.L., Sims, R.C. and T.E. Short. "A Mathematical Model for the Fate of Hazardous Substances in Soil: Model Description and Experimental Results". *Haz. Waste and Haz. Materials 4*:223-240, 1987.

5. Charbeneau, R.J., Weaver, J.W. and V.J. Smith. "Kinematic Modelling of Multiphase Solute Transport in the Vadose Zone". Final Report, Cooperative Agreement CR-813080, Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, Oklahoma, 1989, 108 pages.

6. McGinnis, G.D., Borazjani, H., McFarland, L.K., Pope, D.F. and D.A. Strobel. "Characterization and Laboratory Soil Treatability Studies for Creosote and Pentachlorophenol Sludges and Contaminated Soil". Final Project Report, Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, Oklahoma, 1988, 138 pages.

7. U.S. Environmental Protection Agency. **Test Methods for Evaluating Solid Waste** SW-846, Volumes 1-4 (Third Edition). National Technical Information Service, Springfield, Virginia, 1986.

8. Dutka, B.J. and G. Bitton. **Toxicity Testing Using Microorganisms**. Boca Raton, Florida, CRC Press, Inc., 1986, 395 pages.

9. Liu, D. and B.J. Dutka (Editors). **Toxicity Screening Procedures Using Bacterial Systems**. Marcel Dekker, Inc., New York, 1984, 470 pages.

10. Hermans, J., Busser, F., Leeuwangh, P. and A. Musch. "QSAR's and Mixture Toxicity of Organic Chemicals in *Photobacterium Phosphoreum*: The Microtox© Test". *Ecotox. Environ. Safety 9:* 17-25, 1985.

11. Kamlet, M.J., Doherty, R.M., Veith, G.D., Taft, R.W., and M.H. Abraham. "Solubility Properties in Polymers and Biological Media 7. An Analysis of Toxicant Properties That Influence Inhibition of Bioluminescence in *Photobacterium Phosphorium* (The Microtox Test)". *Environ. Sci. Tech.*: 690-695, 1986.

12. Matthews, J.E. and A.A. Bulich. "A Toxicity Reduction Test System to Assist in Predicting Land Treatability of Hazardous Organic Wastes". In: J.K. Petros, Jr. et al., Editors, **Hazardous and Industrial Solid Waste Testing: Fourth Symposium**. Philadelphia, ASTM/STP 886, 1984.

13. Matthews, J.E. and L. Hastings. "Evaluation of Toxicity Test Procedure for Screening Treatability Potential of Waste in Soil". *Toxicity Assessment 2*: 265-281, 1987.

14. Beckman Instruments, Inc.. *Microtox© System Operating Manual.* Beckman Instruments, Inc., Carlsbad, California, 1982, 52 pages.

15. Sims, R.C. "Loading Rates and Frequencies for Land Treatment Systems in Land Treatment". In: R.C. Loehr and J.F. Malina, Jr., Editors, **Land Treatment: A Hazardous Waste Management Alternative**. Water Resources Symposium 13, Center for Research in Water Resources, The University of Texas at Austin, Austin, Texas 78712, 1986, 370 pages.

16. Huddleston, R.L., Bleckmann, C.A. and J.R. Wolfe. "Land Treatment Biological Degradation Processes". In: R.C. Loehr and J.F. Malina, Jr., Editors, **Land Treatment: A Hazardous Waste Management Alternative**. Water Resources Symposium 13, Center for Research in Water Resources, The University of Texas at Austin, Austin, Texas 78712, 1986, 370 pages.

17. Edgehill, R.V. and R.K. Finn. "Microbial Treatment of Soil to Remove Pentachlorophenol". *Appl. Environ. Microbiol. 45*:1122-1125, 1983.

18. Kennedy, J.B. and A.M. Neville. **Basic Statistical Methods for Engineers and Scientists** (Third Edition). Harper and Row, Inc., New York, 1986, 495 pages.

19. Weber, W.J., Jr. **Physicochemical Process for Water Quality Control**. John Wiley and Sons, Inc., New York, 1972, 640 pages.

20. Chiou, C.T., Porter, P.E. and D.W. Schmedding. "Partition Equilibrium of Nonionic Organic Compounds Between Soil Organic Matter and Water". *Environ. Sci. Technol 17*:227-231, 1983.

21. Sheets, T.J., Crafts, A.S. and H.R. Drever. "Influence of Soil Properties on the Phytotoxicity of s-triazines". *J. Agric. Food Chem. 10*:458-462, 1962.

22. Savage, K.E. and R.D. Wauchope. "Fluometuron Adsorption-Desorption Equlibria in Soils". *Weed Sci. 21*:106-110, 1974.

23. Hamaker, J.W. and J.M. Thompson. "Adsorption". In: Goring and Hamaker, Editors, **Organic Chemicals in the Environment**. Marcel Dekker, Inc., New York, 1972.

24. Rao, P.S.C. and J.M. Davidson. "Estimation of Pesticide Retention and Transportation Parameters Required in Nonpoint Source Pollution Models". **Environmental Impact of Nonpoint Source Pollution**, Ann Arbor Science Publications, Ann Arbor, Michigan, 1980, pages 23-67.

25. McCarty, P.L., Reinhard, M. and B.E. Rittmann. "Trace Organics in Groundwater". *Environ. Sci. Technol. 15*:40-51, 1981.

26. Schwarzenbach, R.P. and J. Westell. "Transport of Nonpolar Organic Compounds from Surface Water to Groundwater". *Environ. Sci. Technol. 15*:1360-1367, 1981.

27. Sawyer, C.N. and and P.L. McCarty **Chemistry for Environmental Engineers** (Third Edition). McGraw-Hill, Inc., New York, New York, 1978, 370 pages.

28. Schellenberg, K., Leuenberger, C. and R.P. Schwarzenbach. "Sorption of Chlorinated Phenols by Natural Sediments and Aquifer Materials". *Environ. Sci. Technol. 18*:652-657, 1984.

29. Zachara, J.M., Alnsworth, C.C., Cowan, C.E. and B.L. Thomas. "Sorption of Binary Mixtures of Aromatic Nitrogen Heterocyclic Compounds on Subsurface Materials". *Environ. Sci. Technol. 21*:397-402, 1987.

30. Murin, C.J. and V.L. Snoeyink. "Competitive Adsorption of 2,4-Dichlorophenol and 2,4,6-Trichlorophenol in the Nanomolar to Micromolar Concentration Range". *Environ. Sci. Technol. 13*:305-311, 1979.

31. U.S. Environmental Protection Agency. "Batch Type Adsorption Procedures for Estimating Soil Attenuation of Chemicals". Technical Resource Document (EPA/530-SW-87-006), Office of Solid Waste and Emergency Response, Washington, D.C. , 1986, 183 pages.

32. Verschueren, K. **Handbook of Environmental Data on Organic Chemicals, Second Edition**. Van Nostrand Reinhold Company, Inc., New York , 1983, 1310 pages.

33. American Society for Testing Materials. **Annual Book of ASTM Standards, Part 19. Soil and Rock, Building Stone**. Philadelphia, Pennsylvania, ASTM D2216, 1982, pp. 338-339.

34. U.S. Environmental Protection Agency, Office of Solid Waste. **Permit Guidance Manual on Hazardous Waste Land Treatment Demonstrations -- Final Version**. National Technical Information Service, Springfield, Virginia , 1986, 98 pages.

35. Burrows, R.D. Letter to R.C. Loehr, December 1986.

36. American Public Health Association. **Standard Methods for the Examination of Water and Wastewater**, 16th Edition. American Public Health Association, Washington, DC, 1985, 1268 pages.

37. Qureshi, A.A., Coleman, R.N., and J.H. Paran. "Evaluation and Refinement of the Microtox© Test". In: D. Liu and B.J. Dutka, Editors, **Toxicity Screening Procedures Using Bacterial Systems**. New York, Marcel Dekker, Inc., 1984, pages 1-22.

APPENDIX A

THE MICROTOX© TOXICITY ASSAY USED IN THIS STUDY

INTRODUCTION

The Microtox© toxicity assay was used for toxicity screening due to its simplicity, rapidity, cost effectiveness, and because the assay procedure had undergone evaluation and standardization for this purpose(12, 13). The test organism is a marine bioluminescent bacteria (*Photobacterium phosphoreum* Strain NRRL B-11177). The Microtox© Model 2055 Toxicity Analyzer System and Microtox© accessories used in this research were obtained from Beckman Instruments, Inc., and Microbics, Inc., Carlsbad, California.

EVALUATION OF CHEMICAL LOSS USING MICROCOSMS

The batch type microcosms used to simulate aerobic soil conditions were 150 mL glass beakers. The chemicals were extracted from the soil microcosms with Methylene Chloride using a Soxhlet extraction apparatus. The extract was concentrated using a Kuderna-Danish apparatus. The final extract was analyzed with a Model 5890 (Hewlett Packard) Gas Chromatographic System.

WATER EXTRACTION OF MICROCOSMS

For the Microtox© analyses, a water soluble fraction (WSF) is needed. The contents of a microcosm were transferred into an amber glass bottle having a Teflon-lined cap and 40 mL (1:4 by soil weight:water volume) of distilled deionized water was added. The soil-water mixture was extracted in a rotary extractor for about 24 hours at 30 rpm at room temperature. Immediately after the extraction, the sample was centrifuged at about 2000 rpm for 15 minutes to separate the WSF. The WSF was vacuum filtered through a 0.45 µm membrane filter. Ten milliliters of the filtered sample were adjusted to 2% NaCl using reagent grade dry NaCl and tested for toxicity. In addition, the pH of the composite sample of triplicates of a chemical loading at a sampling time was measured. The remaining portion of the WSF was stored in a sample vial with minimum head space. The vial was frozen to < -20° C to maintain the integrity of the chemical composition of the sample until further analysis by HPLC.

TOXICITY ASSAY

The toxicity assay was used to: (a) determine the Effective Concentration (EC_{50}) of a chemical solution or of a waste, (b) determine the EC_{50} of the WSF to determine the safe initial chemical loading on soil, and (c) study the toxicity reduction of the WSF.

EC_{50} DETERMINATION

The EC_{50} of a sample is defined as the concentration of the sample required to reduce the initial luminescence of the bacterial suspension by 50% under standard test conditions. To determine the EC_{50} of the chemicals that were evaluated, a solution of a known concentration of a chemical was prepared in distilled deionized water (DDW). The DDW used in the sample preparation did not exhibit any toxicity as determined by the Microtox© assay. The solution was mixed using a magnetic stirrer for 24 hours or until the chemical was completely dissolved. To the extent possible, the solubility of the chemical was estimated from the literature, before attempting sample preparation. The chemical solution was adjusted to 2% Sodium Chloride (NaCl) using either reagent grade dry NaCl or Microtox© Osmotic Adjusting Solution (MOAS -- 22% NaCl solution).

The sample preparation for EC_{50} values that were part of the acceptable loading determination and toxicity reduction studies followed the same procedure.

ASSAY PROCEDURE

This section briefly describes the standard Microtox© toxicity assay procedure. A detailed description of the assay can be found elsewhere (14, 37).

The lyophilized Microtox© bacteria are reconstituted with 1.0 mL of reconstitution solution (ultra-pure water) maintained at 2-4° C in a glass cuvette. Aliquots of 10µL of the reconstituted bacterial suspension are equilibrated for about 15 minutes at 15° C in 0.5 mL of Microtox© diluent (2% NaCl solution) in glass cuvettes. At the end of the equilibration period, the initial luminescence intensity is measured as light units using the Microtox© System. 0.5 mL of the sample and three serial dilutions of the sample are added to the respective cuvettes. The final luminescence readings are taken at the end of the desired exposure time intervals: 5 minutes, 15 minutes, 30 minutes or longer.

The test sample and its dilutions were tested in duplicate. Blank cuvettes containing just the diluent also are read to correct for any shift in the luminescence that may be caused by the bacteria or the machine. From the observed results a dose-response curve is obtained and the EC_{50} value calculated.

CHEMICAL LOADING ON SOIL

The chemical concentration applied to the soil should be within the assimilative capacity of the soil to avoid toxic effects that may limit the microbial degradation of the chemical. The safe chemical loading determination is based on the water soluble fraction (WSF) of the chemical and the toxicity of WSF using the Microtox© assay.

One hundred grams of air-dried soil were weighed into glass jars and mixed with 400 mL (1:4 by soil weight : water volume) of DDW. Immediately, the organic chemical (solution prepared in ethanol) was added to the soil-water mixture, at concentrations of 2x, 5x, 10x and 20x where *x* is the EC_{50} of the chemical. All chemical loadings were prepared in duplicate. The maximum concentration of the ethanol in the water was limited by the required chemical loading and the ease with which the chemical dissolved in ethanol. The maximum concentration of ethanol in water was 2500 to 5000 ppm. The EC_{50} value of ethanol using the Microtox© assay is 30,000-35,000 ppm. Hence, the concentrations of ethanol used in these experiments did not have a significant influence on the toxicity of WSF. The chemical was extracted with DDW for about 24 hours in a tumbler shaker rotating at 30 rpm at room temperature (usually 21-25° C).

After the extraction, the soil-water mixture was allowed to settle for about 20 minutes. About 50 mL of the supernatant was transferred into small glass bottles which were centrifuged at about 2000 rpm for 15 minutes to separate the WSF from the soil particles. The WSF was filtered through a 0.45 µm filter using a vacuum. The filtered sample was osmotically adjusted to 2% NaCl with dry NaCl and analyzed using the Microtox© system. These results were used to indicate the safe lower chemical loading on the soil. The upper limit was set as two times the lower chemical loading.

MICROTOX© ANALYSIS

The sample analysis using the Microtox© system was done as described above. The luminescence readings are converted into toxicity measurements as indicated in the system operating manual(14):

$$G = (I_i * X \div I_f) - 1.0$$

where

I_i = Initial luminescence reading

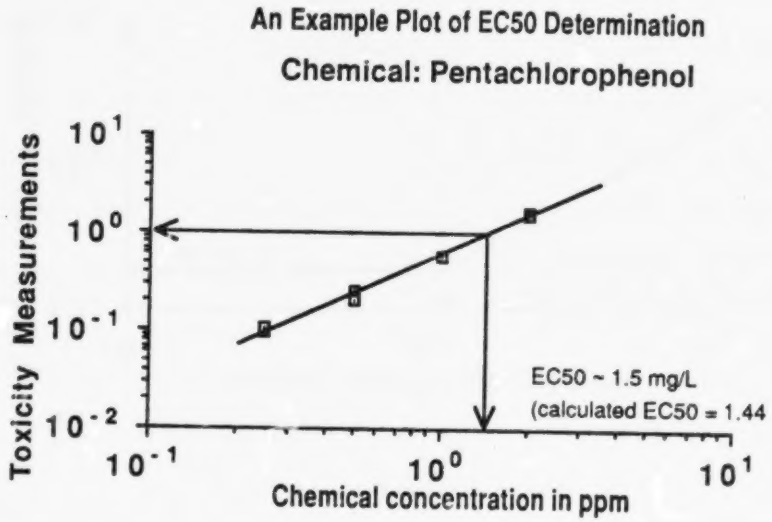
I_f = Final luminescence reading and,

X = Blank Ratio = $I_f \div I_i$ from the blank cuvette luminescence reading

G = Relative toxicity measurement

The concentration of the sample, as ppm or % of the sample solution, is plotted against the toxicity measurements, G, on a log-log scale. The concentration of the sample corresponding to a toxicity measurement of 1.0 is termed the EC_{50} of the sample, as shown below.

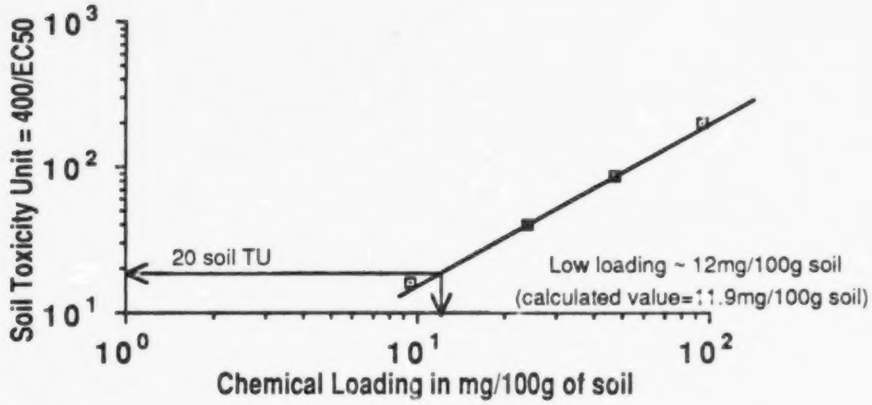
In the chemical loading determination and toxicity reduction study, the EC_{50} of the water extract was converted to soil toxicity units (soil TU) in the following manner(13, 37): $TU = 400 \div EC_{50}$. The chemical loading and the corresponding soil TU were plotted on a log-log graph.



As has been described(13), the point at which the loading rate intercepted the value of 20 toxicity units (TU), i.e., $EC_{50} = 20$, was used as the lower limit, or toxic floor, of the initial loading rate window. The available logic and data(13) indicated that chemicals or waste that exhibited an EC_{50} less than 20 were likely to cause inhibition of the microorganisms in the soil and result in no detoxification. An example plot is shown below.

An Example Plot of Chemical Loading Determination

Chemical: 3-chiorophenol



APPENDIX B

QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

The objective of this study was to evaluate the relative toxicity, degradation kinetics and sorption of specific EPA listed hazardous chemicals and a specific hazardous waste and related chemicals. The data generated consisted of physical and chemical analyses performed by the project personnel. All data and observations were recorded in permanent laboratory notebooks. Replicates and standards were part of the analytical protocol. The following sections describe measures used to determine the accuracy and precision of the measurements.

TOXICITY

Toxicity screening and relative toxicity evaluations were performed using the Microtox© system to establish EC_{50} values for each chemical. Procedures for the Microtox© system outlined in the operation manual were followed(14). EC_{50} values were determined graphically by evaluating duplicate samples at each chemical concentration. To evaluate randomness in toxicity data, EC_{50} data and 95% confidence intervals were reported.

Water soluble fraction (WSF) samples were obtained following procedures outlined in EPA SW-846(7). Duplicate samples of WSF at each chemical concentration were used to graphically determine the chemical loading rate as outlined in Section 3.

DEGRADATION STUDIES

Accuracy and precision of degradation data were monitored routinely. The procedures and analytical techniques included replicate analyses and determination of recovery efficiencies as part of the overall quality control effort. Replicate analysis of three samples at each sampling time avoided basing degradation kinetic results on one data point which could be an outlier.

Illustrative recovery efficiency data are presented in Table B-1. The analytical data were reviewed frequently to discover possible anomalies or omissions. If suspect data were discovered, the raw data used to calculate the results were reviewed and the experiment in question was repeated if necessary. To determine the conformity of the degradation data to zero and first order kinetic models, 95% confidence intervals were determined for all chemicals.

TABLE B-1. ACCURACY DATA: RECOVERY EFFICIENCIES (%) FOR SPECIFIC CHEMICALS AS DETERMINED FROM DAY ZERO DEGRADATION STUDY EXPERIMENTS

Compound	# 1	# 2	# 3	Average	Std. Dev.	C.V.
Texas Soil						
Phenol	97	84	83	88	7.8	8.9
m-Cresol	78	85	81	81 3	3.5	4.3
2-Chlorophenol	73	71	76	73.3	2.5	3.4
2,4,5-Trichlorophenol	99	95	98	97.3	2.1	2.1
Pentachlorophenol	118	117	110	115	4.4	3.8
2,4-Dimethylphenol	84	80	76	80	4.0	5.0
3-Methyl-4-Chlorophenol	89	105	104	99.3	9.0	9.0
p-Nitrophenol	122	101	108	110.3	10.7	9.7
Mississippi Soil						
Phenol	80	75	80	78.3	2.9	3.7
m-Cresol	93	86	84	87.7	4.7	5.4
2-Chlorophenol	27	23	24	24.7	2.1	8.4
2,4,5-Trichlorophenol	110	107	107	108	1.7	1.6
Pentachlorophenol	108	105	106	106.3	1.5	1.4
2,4-Dimethylphenol	83	80	80	81	1.7	2.1
3-Methyl-4-Chlorophenol	99	101	98	99.3	1.5	1.5
p-Nitrophenol	100	116	114	110	8.7	7.9

+ *standard deviation*
++ *coefficient deviation (%)*

Degradation study procedures, extraction and analytical methods, and data analysis procedures were described in Section 5. The day zero percent recovery value for each chemical was assumed to remain unchanged for extractions at later sampling periods.

To understand the typical variation that could occur with the day zero recovery data (chemical recovery efficiencies), multiple extractions of three chemicals representative of those used in this study were conducted. The chemicals were: Phenol, o-Cresol, and 2,4-Dichlorophenol. In each case, each chemical was added to the Texas soil and immediately extracted and analyzed using the procedures presented in Sections 3 and 5. From eighteen to thirty-one extractions and analyses were conducted with these chemicals.

The results that were obtained are shown in Figures B-1, B-2 and B-3. Data in the figures include the average percent recovery, two standard deviations of the data as upper and lower warning limits, and three standard deviations as the upper and lower control limits. Ideally, the data should fall within two standard deviations of the mean. Based on the data in these figures, the recovery efficiencies used in the degradation study calculations (Table 16) fall within satisfactory limits. Since the recoveries for these three chemicals were within satisfactory limits, it was assumed that the recoveries for the other chemicals (Table 16) also were within satisfactory limits.

ADSORPTION STUDIES

Procedures used to obtain the adsorption data were described in Section 6. Isotherms were determined using soil-chemical mixtures with different initial concentrations of chemical. Replicate samples of the low and high concentrations were run. Precision was monitored using replicate data points. These replicate results were averaged and standard deviations and corresponding coefficients of

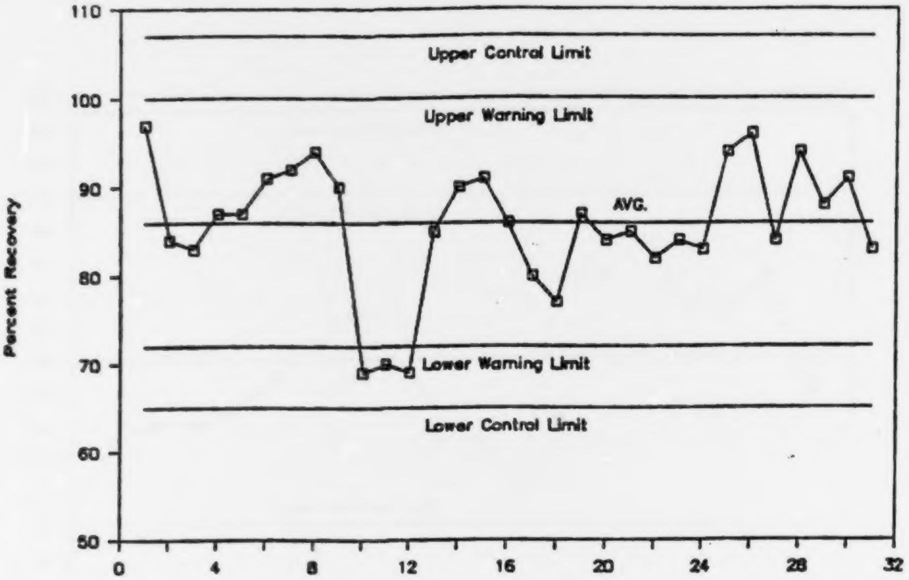


Figure B-1. Representative Recovery Data for Phenol in Texas Soil

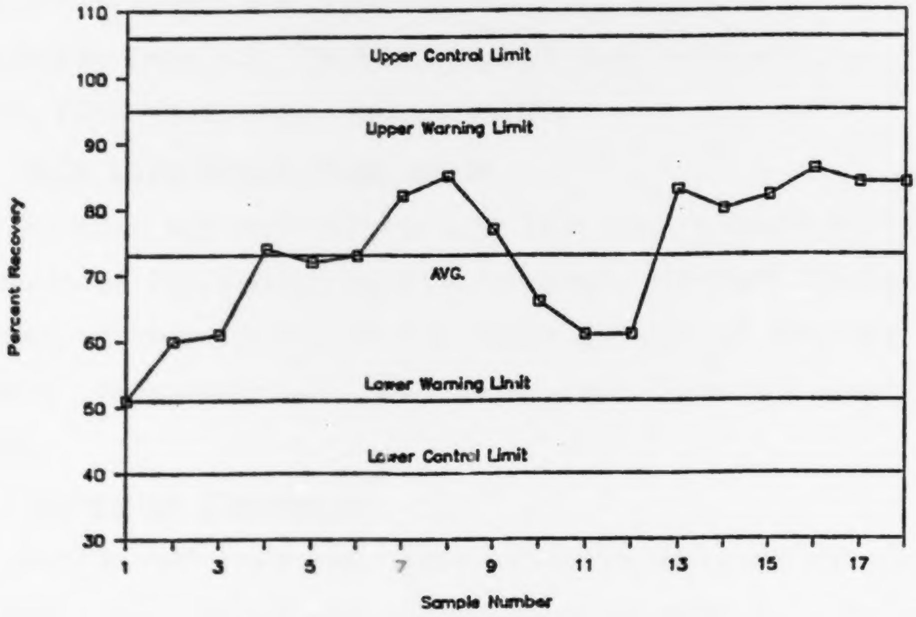


Figure B.2. Representative Recovery Data for o-Cresol in Texas Soil

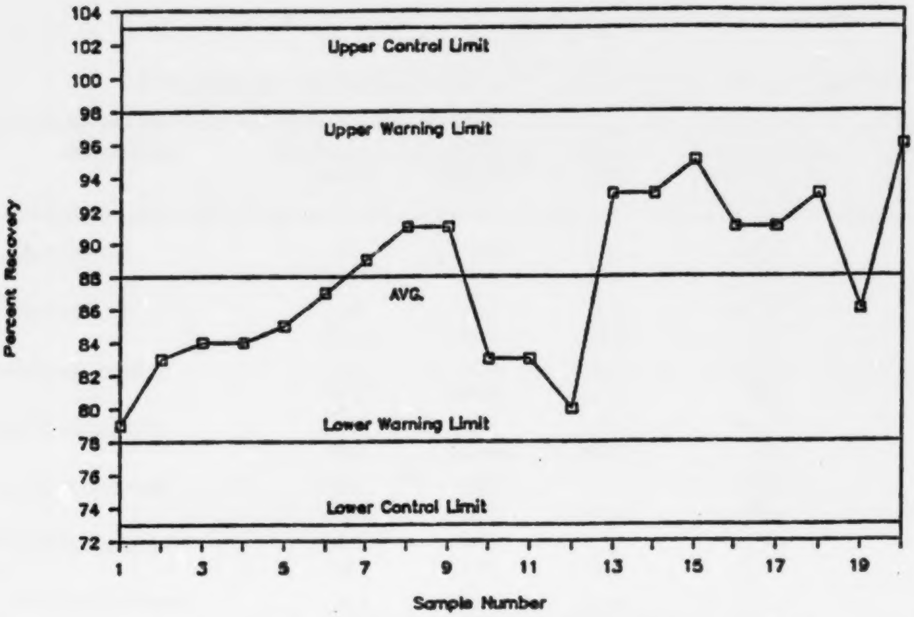


Figure B-3. Representative Recovery Data for 2,4-Dichlorophenol in Texas Soil

variation (CV) for each sample pair were calculated. Illustrative data are presented in Table B-2 for Texas soil. The results indicated close agreement among duplicates.

QA/QC FOR ANALYTICAL INSTRUMENTS

Daily Logs of Instrument Usage

Individual logs were maintained for each major analytical instrument (gas chromatograph, high pressure liquid chromatography, Microtox©. The log contained information pertaining to instrument conditions and analyses performed. Unusual events or circumstances were noted and reported if instrument performance was affected.

Calibration Procedures

Prior to each day's analysis the instruments were calibrated using known standards. Both internal and external standards were used depending upon application. Instrument responses of greater than 10% of that expected for standard

TABLE B-2

PRECISION ANALYSIS DATA+: TEXAS SOIL SORPTION DATA

CHEMICAL	SAMPLE A (mg/l)	SAMPLE B (mg/l)	MEAN (mg/l)	STANDARD DEVIATION (mg/l)	COEFF. OF VARIATION (%)
3-Chlorophenol	9.7	10.0	9.9	0.21	2.15
	5540	5550	5545	6.2	0.11
4-Chlorophenol	2.7	2.7	2.7	0.00	0.00
	5810	6250	6030	313	5.19
2,4-Dichlorophenol	3.9	3.9	3.9	0.00	0.00
	1180	1260	1220	56	4.59
2,5-Dichlorophenol	4.4	5.2	4.8	0.57	11.8
	1980	2040	2010	42	2.14
2,6-Dichlorophenol	16.3	16.8	16.6	0.35	2.15
	1380	1480	1430	65	4.58
3,4-Dichlorophenol	4.3	5.3	4.8	0.71	14.7
	659	659	659	0.00	0.00
2,4,5-Trichlorophenol	24.7	24.9	24.8	0.14	0.57
	508	508	508	0.00	0.00
2,4,6-Trichlorophenol	21.8	22.1	22.0	0.21	0.97
	298	298	298	0.00	0.00
Pentachlorophenol	0.8	0.8	0.8	0.00	0.00
	4.5	4.6	4.6	0.07	1.55
2,4-Dimethylphenol	33.4	34.0	33.7	0.42	1.26
	4770	4950	4860	133	2.75
2--Methyl,4-Chlorophenol	7.2	7.8	7.5	0.42	5.66
	1730	1780	1760	37	2.11
3--Methyl,4-Chlorophenol	5.8	5.8	5.8	0.00	0.00
	1930	1950	1940	11	0.59
3-Methyl,6-Chlorophenol	72.6	67.8	70.2	3.4	4.83
	2570	2680	2630	75	2.85
p-Nitrophenol	6.3	6.5	6.4	0.14	2.21
	3520	3720	3620	144	3.99
2,4-Dinitrophenol	5.6	6.0	5.8	0.28	4.88
	445	463	454	12.3	2.71
4,6-Dinitro-o-Cresol	49.4	52.0	50.7	1.84	3.63
	112	120	116	5.8	4.97
Thiourea	42.4	43.5	43.0	0.78	1.81
	4380	4700	4540	225	4.96

+ *The two values for each chemical represent low and high concentrations used in the sorption studies.*

solutions required setting new chemical standards and/or making appropriate adjustments to the instrument. This action was the responsibility of the project analyst.

All reagents for chemical analysis were prepared using analytical reagent grade (AR) chemicals. For all analyses requiring reagent water, organic free DDW was used.

Data calculation, manipulaitons and analysis were performed using calculators and computers. Computer programs used for data reduciton and analysis were validated before use.

APPENDIX C

VOLATILIZATION ESTIMATES

The results of the degradation studies (Section 5) represent the overall chemical loss that occurred in the soil microcosms. While the main loss mechanism was expected to be microbial degradation, it was possible that chemical degradation, hydrolysis and volatilization could have contributed to the removals that were observed. Most of the chemicals evaluated had low vapor pressures and volatilization losses were assumed to be negligible.

However, several chemicals such as Methanol and 1-Butanol had higher vapor pressures, and it was possible that appreciable volatilization losses could have occurred during the degradation studies. To evaluate this possibility, a series of simple experiments was conducted to estimate the amount of volatilization that might occur.

These experiments consisted of adding the chemicals to two soil microcosms, one of which had a constant air sweep to remove any volatilized chemical, and the other of which did not have any air sweep. The experiments were conducted for two and twenty-four hours. These times were used because the greatest volatilization potential occurred when the chemical concentration in the soil was the highest, i.e., at the beginning of a study. Twenty-four hours was the longest period that could be used since degradation also would be occurring during the experiments, thus decreasing the chemical concentration.

Texas soil was used for these experiments. The chemicals were added to the soil to achieve a concentration of about 1000 mg/kg which was close to but higher than the concentrations of Methanol and 1-Butanol used in the degradation studies. The air sweep was held constant at 100 ml/minute during the experiments. The results are presented in Table C-1.

TABLE C-1. LOSS OF METHANOL AND 1-BUTANOL IN VOLATILIZATION EXPERIMENTS

Condition	Time Period (hours)	Chemical Added (mg)++	Chemical Remaining (mg)
Methanol			
no air sweep	2	40	25.0
continuous air sweep+	2	40	23.5
1-Butanol			
no air sweep	2	40	34.2
continuous air sweep+	2	40	34.8
no air sweep	24	40	35.2
continuous air sweep+	24	40	14.2

+ *A constant air flow of 100 ml/min was maintained over the surface of the microcosms throughout the noted time period.*

++ *This amount of chemical was added to 40 grams of soil resulting in an initial soil concentration of 1000 mg/kg.*

The data indicate that there was little change due to the air sweep and little enhanced volatile loss during a two-hour time period. However, over a twenty-four hour period the continuous air sweep did increase the loss of 1-Butanol.

The driving force for volatilization is related to the concentration gradient at the boundary layer (soil surface) established by the concentration in the soil and the concentration in the air layer immediately above the soil. With an air sweep, any volatile constituents in the air layer above the soil are continuously removed, the concentration gradient will be maximum, and the volatilization potential will be the greatest. In the degradation experiments, quiescent conditions were maintained and no air sweep was used. Under quiescent conditions, if any volatilization occurs, the concentration above the soil builds up, the concentration gradient is less, the volatilization potential is the least, and the volatile flux from the soil is suppressed.

For this reason, even for chemicals in this study with a high vapor pressure, little volatilization was expected under the conditions used for the degradation experiments.

APPENDIX D

PUBLICATIONS

In addition to this report, a technical report and several technical articles, two M.S. theses, one Ph.D. dissertation and several presentations have resulted from this research. These are:

TECHNICAL REPORT

One of the objectives of this research effort was an assessment of the terrestrial and aquatic bioaccumulation that could result by the chemicals evaluated in this study. The following draft report was submitted to the EPA project officer, Mr. John E. Matthews:

Loehr, R.C. and R. Krishnamoorthy, "Bioaccumulation Potential of Designated Hazardous Organic Chemicals", July 1987.

The report was reviewed and accepted by the project officer and therefore is not included in this final report.

TECHNICAL ARTICLES

Loehr, R.C. and R. Krishnamoorthy, "Terrestrial Bioaccumulation Potential of Phenolic Compounds", *Hazardous Waste and Hazardous Materials 5*: 109-120 (1988).

Nam-Koong, W., Loehr, R.C. and J.F. Malina, Jr., "Kinetics of Phenolic Compounds in Soil", *Hazardous Waste and Hazardous Materials 4:* 321-328 (1988).

M.S. THESES

Yoon, C.G., "Multisolute Adsorption of Toxic Organic Compounds Onto Soil", The University of Texas at Austin, May 1988.

Dasappa, S.M., "Detoxification and Immobilization of Chlorophenols in Soil", The University of Texas at Austin, September 1988.

PH.D. DISSERTATION

Nam-Koong, W., "Removal of Phenolic Compounds in Soil", The University of Texas at Austin, May 1988.

PRESENTATIONS

Nam-Koong, W., Loehr, R.C. and J.F. Malina, Jr., "Removal of Phenolic Compounds in Soils", Texas Water Pollution Control Association Conference, Corpus Christi, Texas, June 1987.

Dasappa, S.M. and R.C. Loehr, "Detoxification and Immobilization of Chlorophenols in Soil", Texas Water Pollution Control Association Conference, Houston, Texas, June 1988.

Nam-Koong, W., Loehr, R.C., and J.F. Malina, Jr., "Removal of Phenolic Compounds in Soil", Joint CSCE-ASCE National Conference on Environmental Engineering, Vancouver, BC, Canada, July 1988.

Dasappa, S.M. and R.C. Loehr, "Detoxification and Immobilization of Chlorophenols in Soil", 61st Annual Conference, Water Pollution Control Federation, Dallas, Texas, October 1988.

Nam-Koong, W., Loehr, R.C. and J.F. Malina, Jr., "Effects of Mixture and Acclimation on Removal Rate of Phenolic Compounds in Soil", 61st Annual Conference, Water Pollution Control Federation, Dallas, Texas, October 1988.